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ILLINOIS BIOLOGICAL MONOGRAPHS

VOLUME XIV

PUBLISHED BY THE UNIVERSITY OF ILLINOIS

URBANA, ILLINOIS

EDITORIAL COMMITTEE

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JOHN THEODORE BUCHHOLZ
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No. 1

PUBLISHED BY THE UNIVERSITY OF ILLINOIS
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JOHN THEODORE BUCHHOLZ

FRED WILBUR TANNER

CHARLES ZELENY

THE DEVELOPMENT OF THE PECTORAL LIMB OF NECTURUS MACULOSUS

WITH ELEVEN PLATES

BY
HSIN KUO CHEN

CONTRIBUTION FROM THE ZOOLOGICAL LABORATORY OF THE
UNIVERSITY OF ILLINOIS
No. 465

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I. INTRODUCTION

Previous work on the development of the chiropterygious type of limb in vertebrates has been carried on in connection with various problems, which may be divided for convenience into three classes:

(1) The earliest papers dealt chiefly with the origin of the primordial limb bud and its relation to the myotomes. The results obtained, however, vary with the different investigators, and on the basis of the results obtained, the workers may be divided into two groups. The first group, including Goette ('75), Jordon, ('88), Kaestner ('93), Field ('94), Mollier ('95), and Tschernoff ('07) thought that the myotomes take part in the formation of the limb bud either in the form of processes as the muscle buds in fish, or by way of cell migration as in the case of *Bombinator*, described by Goette. The second group of investigators, including Paterson ('88), Harrison ('95, '18), Byrnes ('98), Lewis ('10), and Detwiler ('18), claimed that both observations and experimental methods show that the limb arises as a thickening of somatopleure and is in no way derived from the myotomes or their processes. None of these investigators have attempted to trace the limb development as far as the stage where it is comparable with adult conditions.

(2) Considerable work has been done recently by experimental methods in analyzing the factors which determine the differentiation of the limb bud, the change of symmetry, and the formation of the nerve plexus in transplanted limbs. Investigation in this field has been carried on chiefly by Harrison and his students (Harrison '07, '15, '16, '18, '21, '25; Detwiler '18, '19, '20, '22; and Swett '23, '26).

(3) A new theory concerning the development of the limb musculature in tetrapods has been advanced recently by Romer ('27). According to his theory the muscles of the chiropterygious type of limb may be arranged into two groups, one dorsal (dorso-medial) and one ventral (ventro-lateral). These groups of muscles may be homologous with, or derived from, the two opposed masses of muscles present in the paired fins of fish. This theory was at first based upon his comparative studies of several primitive adult forms of tetrapods ('22, '24), and was later supported by his embryological work on the development of the thigh musculature of the chick ('27).

It was suggested to the writer by Professor Waldo Shumway that a study of the development of the limb in one of the lowest tetrapods, from its earliest appearance to the stage at which it attains the adult form, might give us a better understanding of the relations of the myotomes to the early formation of the limb bud, and the homology of the appendicular musculature with the two opposed masses of muscles in the fish

fin from the embryological point of view. *Necturus* was chosen as the subject for this investigation because of the accessibility of the material. The results obtained in this investigation are presented in the following order: (1) the formation of the primordial limb bud and its relations to the myotomes; (2) the differentiation of the limb bud, in which the primordia of the skeleton, musculature, and nerve plexus are laid down; and (3) the further development of the primordia which ultimately attain the conditions similar to that of the adult.

This work was undertaken under the supervision of Professor Waldo Shumway, to whom I am indebted for advice and criticism. I also wish to express my gratitude to Dr. H. W. Hann for helpful suggestions; to Dr. A. R. Cahn for his kindness in placing a large collection of preserved specimens at my disposal; and to my wife for her ever-ready help during the completion of this investigation.

II. MATERIAL AND METHODS

The embryos and larvae of *Necturus maculosus* were collected at Oconomowoc Lake, Wisconsin, in the summer of 1925. They were killed and fixed in Smith's fluid which was prepared according to the following formula: potassium bichromate .5 gram, glacial acetic acid 2.5 cc., formalin 10 cc., and distilled water 75 cc. The specimens were later preserved in 70% alcohol until ready for use. Serial sections 20 μ in thickness were cut in three different planes by means of the paraffin method. The animals that were sectioned ranged from 9 mm. in length where the primordium of the limb bud is just discernible, to 34 mm. where the structures of the limb are comparable with the adult stage. The sections were stained in a saturated aqueous solution of basic fuchsin for five minutes, and after being rinsed in water, were transferred to equal parts of a saturated solution of indigo-carmin and a saturated solution of picric acid for twenty minutes or more until the sections appeared green (Shumway '26). From this solution the slide was passed rapidly from 70% alcohol to the pure xylol where a bright green indicated that the tissues were well differentiated. Otherwise the sections were decolorized in acid alcohol and restained. In a well-stained section, the following color differentiation appears: precartilage, bright blue; cartilage, deep blue; muscle, grass green; nerve, dark purple to light gray; connective tissue, bluish gray; blood corpuscles and mitotic figures, deep red.

The following table shows the specimens that were sectioned by the writer with the indication of their approximate age and number of somites according to the tables of Eycleshymer and Wilson ('10).

<i>Length in mm.</i>	<i>Age</i>	<i>Somites</i>
9 Trans.....	23 days 10 hrs.	20-22 pairs
10 Trans.....	24 days 22 hrs.	23-24 pairs
12 Trans.....	28 days 2 hrs.	28-29 pairs
13 Trans. (3 series)	30 days 8 hrs.	30-31 pairs
Sagit.		
Front.		
14 Trans.....	32 days 10 hrs.	31-32 pairs
Sagit.		
Front.		
15 Trans.....	34 days 12 hrs.	32-34 pairs
Sagit.		
Front.		
16 Trans.....	36 days 16 hrs.	36-38 pairs
Sagit.		
Front.		
17.5 Trans.....		
18 Trans.....	40 days 2 hrs.	44-46 pairs
Sagit.		
Front.		
19 Trans.....	43 days	48-50 pairs
Sagit.		
Front. (2 series)		
20 Front.	46 days 2 hrs.	50-55 pairs
21 Trans.....	49 days	
Sagit.		
Front. (2 series)		
23 Trans.....	55 days	
Sagit.		
Front.		
27 Front. (2 series)	67 days	
32 Trans.....	87 days	
Front.		
34 Trans.....	97 days	
Front.		

In addition to the above sections the writer has had access to the large collection of slides of *Necturus maculosus* used by Eycleshymer and Wilson in preparing their important monograph on "Normal plates of the development of *Necturus maculosus*" ('10). From all these slides a careful study of embryos and larvae of different stages was made. All drawings of structures at their important developmental periods were outlined by the aid of the camera lucida. Besides the sections, Born's method of wax reconstruction was also employed to study the developing limb as a whole. This facilitates the identification of the tissues separated by different sections, and furnishes a clear picture concerning the degree and extent of the development of various structures in relation to one another. Models prepared by DeBruine ('28) were compared with those made especially for this investigation.

The terminology applied to the skeleton and musculature of the

limb in *Amphibia* varies with the different authors. In order to avoid confusion the nomenclature employed by Wilder ('03, '12) is adopted in the following descriptions.

III. THE PRIMORDIAL LIMB BUD

1. STRUCTURE OF THE UNDIFFERENTIATED MESENCHYME

The earliest stage at which the mesenchyme cells appear in the limb is in the embryo 9 mm. in length (Fig. 1). They are found on the ventro-lateral side of the myotome and scattered among the numerous yolk granules, which vary in size but are quite uniformly distributed throughout the whole region. The form of the cell (Fig. 7) varies somewhat, the round and oval being the most common, with oblong and polyhedral ones occurring occasionally. The main bulk of the cell is composed of nucleus. The membrane of the nucleus is well defined, and terminating in it are the delicate chromatin threads. The threads form a fairly regular meshed reticulum. Imbedded in it are several nucleoli and many small chromatin bodies. At this stage, the mitotic figures are rarely observed. The whole bud appears as a slightly elevated ridge at the surface of the body wall. In embryos between 10 and 11 mm. in length (Figs. 2, 3), the mesenchyme cells increase slightly in size and number. Their chromatin network is more clearly shown and associated with it are several deeply stained karyosomes. The majority of the cells are now oval in shape and only a small number of them are round or polyhedral. The yolk granules, as before, are different in form—round, oval, and quadrate—and are still thickly and evenly distributed in the limb region. The general outline of the limb is not much different from that of the preceding stage.

As the development proceeds, the limb bud is more prominently marked. At the 12 mm. stage (Fig. 4), the mesenchyme cells form a rather compact mass and push out from the surface of the body wall. At this time, the decrease in the number of yolk granules commences, and the mitotic figures are frequently seen, indicating the beginning of active growth. The structure of the nuclei, however, does not widely differ from that just described.

In the succeeding stage, great changes occur in the limb region. In an embryo 13 mm. in length the yolk granules have almost entirely disappeared and the mesenchyme cells tend to collect together. A great many of the nuclei are still oval, but an increase in the number of cells and the numerous mitotic divisions observed at this stage mark the period of active growth.

This period of active growth extends to the next stage when the embryo is 14 mm. in length (Figs. 8, 9), when a noticeable change occurs involving the elongation of many nuclei into cylindrical form. Along with this change, the chromatin network is very clearly revealed, and its meshed reticulum becomes larger with a considerable increase in the number of threads. Among the meshes there are several nucleoli seen as small deeply staining bodies.

Following this stage, the mesenchyme cells in the limb bud, on account of the active multiplication, show a slight tendency to divide into peripheral and central groups (Fig. 10). This phenomenon is marked by the growth of the blood vessels in the region between these groups. In a transverse section through the limb area of a 15 mm. embryo, the spaces between these groups occupied by blood vessels are quite noticeable. The cells on the peripheral or the outer zone as seen in transverse sections are usually cylindrical with their longest axes radiating from the center of the limb, while those in the central or inner region are commonly oval in shape. Externally these mesenchyme cells are inclosed by the epidermis, which is two layers of cells in thickness.

Through this period of growth, the mesenchyme cells in the central area begin to differentiate into cartilage, and those belonging to the peripheral part later give rise to the muscles. The details of these processes will be taken up in their respective sections.

2. RELATION OF THE MYOTOMES TO THE ANTERIOR LIMB BUD

The formation of myotomes takes place much earlier than that of the anterior limb bud. In embryos with about twenty pairs of myotomes (9 mm. Fig. 1), the primordium of the limb bud becomes discernible as a thickening of the somatopleure below the myotomes. It consists of an aggregation of a few mesenchyme cells embedded among the numerous yolk granules and covered externally by a layer of epidermis. The ventral border of the myotomes in the limb region as seen in transverse sections is somewhat rounded, extending to the level of the center of the notochord without showing any indication of the presence of their ventral process. The cells in the myotomes are commonly round or oval, and packed together with yolk granules. At this stage, the process of fibrillation has already begun, and the delicate threads are easily found, especially at the ventral side of the myotome. Soon after the appearance of the anterior limb bud, the rudiment of the pronephros may be seen in the region medial to the dorsal part of the limb bud. The pronephric tubules are composed of a few cells densely laden with yolk

granules, and show a somewhat tubular arrangement. Thus at the outset, the limb bud and the pronephros are closely associated.

In a slightly later stage, the myotomes in the anterior trunk region grow downward. In the embryo with about 24 pairs of myotomes (10 mm.), the myotome processes begin to appear on the ventral outer-border and soon extend to the level of the lower margin of the dorsal aorta (Fig. 2). The cells in the ventral processes become elongated in the direction of their growth. Simultaneously with the development of the ventral processes, the pronephroi become more definite in form, with their cells arranged into tubular outline inclosing a cavity in the center. The yolk granules previously scattered around are now confined in the walls of the tubules. In a transverse section of a 10 mm. embryo through the limb region, the pronephric tubules are shown on the dorso-lateral border of the somatopleure a little below the distal end of the myotome process. Hence in the next stage, when the ventral processes elongate farther downward, they meet the pronephric tubules and grow along their lateral side, medial to the limb bud. This is shown in a transverse section of an 11 mm. embryo (Fig. 3), in which the tips of the myotome processes have already grown over the pronephric tubules and entered the dorsal region of the anterior limb bud. This indicates that the close proximity of the myotome processes and the limb bud is brought about by the outgrowth of the pronephric tubules, which at this stage elongate at right angles to the direction of the growth of the ventral processes. In spite of their close association with the mesenchyme cells of the limb bud, the cells of the myotome processes can be easily distinguished by their arrangement in double strands with the longest axes of their nuclei lying dorso-ventrally in the direction of their growth, while the cells in the limb region do not follow any definite arrangement. This characteristic difference in the cells of the myotome processes and of the limb bud make it easier to determine whether or not there is any migration of cells from the former to the latter. Close examinations have not disclosed any mingling of cells from these two separate sources, nor is there any indication of migration of cells from the myotome derivatives to the limb bud. This strongly suggests that at least in the group of urodeles the evidence supporting the assumption that the mesenchyme in the primordial limb is derived from the myotomes is far from being conclusive.

The character of the independence of the limb bud from the myotome derivatives is more clearly shown in the succeeding stage when the myotome processes have reached into the ventral base of the limb bud, and appear in transverse section as a long band consisting of two or three columns of nuclei closely packed together. Their well-defined gen-

eral outlines, together with the dorso-ventrally elongated nuclei and deep-staining capacity, make them readily recognizable from the surrounding structures. In a transverse section of a 12 mm. embryo (Fig. 4), it is seen that between the medial margin of the limb and the ventral myotome process there exists a clear space in which no trace of cells is found. Such clear space naturally indicates the separation and independence of the limb bud from the myotome derivatives. The assumption that there is a migration of cells from such a well-isolated myotome process to the limb region without any trace of cells connecting them is hardly probable. On the other hand, it does show that the cells in the limb bud multiply and differentiate in situ, as manifested by the frequent occurrence of mitotic figures among them, and by their increase in number and size at the time when the influence of the myotome processes does not reach to the limb region. This situation becomes more conspicuous as development progresses.

In embryos between 13 and 14 mm. in length (Fig. 9), the pronephric tubules elongate greatly in the direction of the limb bud and become convoluted. By their excessive growth, they force their way through the ventral myotome processes and come to lie near the base of the limb. In this manner, the ventral processes are cut off from their respective myotomes. Posterior to the pronephric region, the connection between the myotomes and their processes still persists. In the meantime, the cells in the limb region enter the period of active proliferation and numerous mitotic figures may be seen. As a result the limb bud grows out on the surface of the body wall. On the other hand, the isolated ventral myotome processes show little sign of cell division, and mitotic figures are only occasionally observed. The characteristic dorso-ventral elongation of the cell persists a while, but is soon lost. The myotome processes appear now as a thick band of cells bordering on the outer margin of the coelom at the ventral base of the limb. A comparison of the numerous cases of cell division in the limb region, with that of a few instances occurring in the ventral processes, can hardly convince one that the source of the limb material depends upon the myotome derivatives. Instead, it indicates that the limb-forming substance is differentiated in situ rather than it is derived from the myotome processes.

In a transverse section of a 14 mm. embryo (Fig. 9), it is shown that the cells in the limb bud are crowded together at the central part, but become thinner toward the base or proximal region. The cell divisions are very active in the area where the cells are most crowded. The isolated ventral process, as shown in the section, extends from the middle part of the medial margin of the limb, to its ventral base. Its general form is so well defined and distinguished that it does not show any feature of

intermingling with the nearby mesenchyme cells. The cells in the process are either oval or elongated and mixed together with yolk granules. They are confined within the boundary of the process. The region of the limb close to the ventral process is filled with only a few loosely distributed mesenchyme cells. Mitotic divisions occur less frequently here than farther away. Comparing the conditions found in the myotome processes and in the region close to them with those seen more distant from them, one is justified in saying that the myotome processes do not take part in the formation of the limb. In the same section, the excessive growth of the pronephric tubules is clearly shown. They become convoluted and push out farther than the outer lateral margin of the myotome. The myotome processes after being constricted off by the pronephric tubules are pressed outward closely against the base of the limb.

In subsequent stages (Figs. 10, 11), differentiation of the limb bud and the final fate of the myotome derivatives conclusively show that they are independent of each other. At the time when the blood vessels appear in the limb region, the cells in the limb bud multiply rapidly and begin to separate into the peripheral and central groups. The peripheral cells later give rise to the muscles, while the central ones form the first center of chondrification. During this period of remarkable changes in the limb region, the isolated ventral myotome processes gradually increase in bulk and grow downward along the outer margin of the somatopleure. In an embryo 17 mm. in length when the humerus enters the period of chondrification, the process of fibrillation has taken place in the myotome processes which are soon transformed into the definite ventral muscles of the body wall along the outer border of the coelom.

The tracing of the ventral myotome processes from their earliest appearance to the stage at which the definitive structures are derived from them warrants the foregoing statement that the limb-forming mesenchyme is differentiated *in situ* and is in no way derived from the myotomes or their processes. The evidences supporting this statement may be summarized as follows:

- (1) The mesenchyme cells of the primordial anterior limb bud are found as the thickening of the somatopleure before the appearance of the ventral myotome processes.

- (2) During development, the pronephric tubules elongate at right angles to the direction of the growth of the ventral myotome processes and press the latter closely against the medial side of the limb bud. The proximity of the limb bud and the myotome processes is merely due to the excessive growth of the pronephric tubules.

- (3) During their close contact with the limb bud, the myotome processes are distinguished from the surrounding mesenchyme cells by

their dorso-ventrally elongated nuclei, by their well-defined general form, and by the existence of a space between the ventral processes and the base of the limb bud. No indication of a migration of cells is found.

(4) When the ventral processes are cut off from their respective myotomes, they grow downward along the outer border of the coelom and later transform definitely into the ventral muscles of the body wall.

IV. DIFFERENTIATION OF THE LIMB BUD

1. FORMATION OF CARTILAGE

A. Differentiation of Chondroblasts and Intercellular Matrix

It has been mentioned in the previous sections that in the early stage the limb bud is composed of a mass of undifferentiated mesenchyme cells extending from the third to the fifth myotomes (Figs. 5, 6). The first indication of the differentiation of chondroblasts takes place in a slightly later stage by a condensation of the cells in the center of the limb bud (Fig. 11). This condensed mass of cells forms the first center of chondrification and represents the blastema of the future humerus. The cells in the blastema possess at first the same form as the undifferentiated mesenchyme, but soon they become elongated. In a frontal section of a 16 mm. embryo through the limb region (Fig. 12), it is seen that the elongated cells at the center of the condensed mass are arranged in transverse rows at right angles to the long axis of the cell mass. At the periphery of the condensed mass, the embryonic chondroblasts merge imperceptibly into the surrounding undifferentiated tissue. From this center of condensation, growth of the limb skeleton proceeds both proximally and distally. Proximally, the cells in the girdle region are soon converted more or less simultaneously into the blastema of the future girdle. Distally, at a slightly later stage, centers of chondrification appear respectively in the ulna and radius. In embryos 18 mm. in length (Figs. 15, 16, 17), rudiments of all parts of the limb skeleton become distinguishable.

Following this incipient condensed stage, the next step in the development of cartilage involves the differentiation of the intercellular matrix. This process is first indicated by the appearance of narrow spaces among the condensed cells. At its beginning, this ground substance is stained light blue by picro-indigo-carmin and its structure is indistinguishable. It appears as a layer of homogeneous, transparent substance over the surface of the closely aggregated chondroblasts. The character of the matrix, however, becomes clearer and clearer in the later stages as it

increases in staining capacity and the chondroblasts move apart from one another. It is now stained deep blue and forms the distinct capsule surrounding each cell. As development progresses, the matrix increases in thickness through the constant secretion of the ground substance and is later clearly revealed as a close network of fine fibers. The assumption made by the early investigators that the matrix of the hyaline cartilage is made of a simple homogeneous material now becomes untenable.

B. Growth of Cartilage

During the period of rapid development, the cartilage increases in length and thickness. This is brought about by both interstitial and appositional growth. By interstitial growth, the cells within the cartilage increase by mitosis. In many bases, after division, two of them are found in the same capsule. When they move apart, a partition, very thin at first, appears between them. These daughter cells may soon divide again. As the cell divisions go on, the new ground substance is constantly laid down, and the cells tend to push themselves apart from one another. In addition to this process of development, the cartilage also increases in size by appositional growth through the formation of new cartilage over the external surface. As it has been mentioned in previous sections there is a zone of undifferentiated cells around the peripheral region of the cartilage. These cells are usually in the process of active cell division. Those nearest to the cartilage first undergo the process of chondrification by which they are transformed into chondroblasts and incorporated into the cartilage. This process constantly takes place during the period of rapid development, and the new cartilage is continuously differentiated and added to the surface of the old layer.

Along with its growth in length and thickness, the cartilage simultaneously differentiates into three zones of cells (Fig. 22). Their mode of development is closely similar to that observed by Fell ('25) in the case of the fowl. In *Necturus*, the earliest stage at which the differentiation of the three zones of cells takes place is in the embryo about 19 mm. in length. The characteristic formation of these zones is most easily seen in a frontal section of the limb. The first zone of cells occurs in the center, or diaphysis, of the cartilage. The cells are characterized by their polyhedral shape with large lacunae and thickened capsules. Some cells in this zone are shrunken, manifesting the tendency toward degeneration. The second zone consists of longitudinally flattened cells lying on both sides of the first zone showing the process of active division. The third zone is confined to the epiphysial region in which the cells are distinguished from the others by their round or polyhedral outline. They are smaller in size and closely arranged in an elliptical form. In this

region, mitosis is most active. Between the first zone or toward the end of diaphysis and the beginning of epiphysis are found several elongated cells arranged in a transverse line.

During the later stages of development several changes occur in these three zones of cells (Fig. 26). The changes are most pronounced in the older larvae. In zone one, especially at the center of the diaphysis, the matrix appears as a piece of spongy network with heavily thickened capsules. The cells embedded in the capsules become greatly shrunk—which results in the formation of lacunae. In zone two the formation of matrix begins among the flattened cells, where at first numerous slender strands are formed which finally become dense and possess the same appearance as those in zone one. In zone three, the epiphysial region is now seen as a close network with numerous cells distributed within the dense matrix. The cells in this region now change their elliptical arrangement and take on the concentric configuration. This is most noticeable in the head of the humerus which shows a sort of ball-like form fitting into the cavity of the girdle.

C. Regional Chondrification of the Limb Skeleton

Starting from the first center of chondrification, it has been found that the earliest blastema formed from the undifferentiated mesenchyme represents the rudiment of the future humerus. At first, it is simply a mass of condensed cells with uniform caliber throughout. As soon as it enters the period of chondrification, the differentiation of diaphysis and epiphysis commences (Fig. 15). The epiphysis, or the extremity of the humerus, is comparatively broad with its surface surrounded by undifferentiated cells, but is demarcated from them by a layer of flattened chondroblasts. When the embryo is about 20 mm. in length, the formation of three zones of cells as previously mentioned takes place.

During the rapid growth of this cartilage, rotation of the limb occurs. Before the chondrification of the humerus, the whole limb bud grows out from the body surface at right angles to the long axis of the embryo and is marked with two borders and two surfaces (Fig. 11). The anterior or the preaxial border directed toward the head of the embryo corresponds to the radial border of the adult tetrapod limb. The posterior or postaxial border directed toward the tail corresponds to the ulnar border of the adult. The upper surface facing dorsally in relation to the embryo is known as the extensor surface. The lower surface turned ventrally is known as the flexor surface. During the process of rotation, the radial or preaxial border becomes ventral, directed toward the body. The ulnar or the postaxial border becomes dorsal, directed toward the cephalic end. The whole limb inclines posteriorly with the flexor sur-

face of the hand pressed against the body. Along with the rotation of the limb, a pronounced bending of the humerus takes place. In a frontal section of the limb at this stage, it is seen that the convex aspect of bending is directed toward the ulnar border, and its concave aspect toward the radial side. Through the formation of this bending, a constriction appears between the diaphysis and epiphysis, making their division more clearly marked.

The next step of chondrification occurs in the girdle region in which three centers arising more or less simultaneously are usually distinguishable (Fig. 18). The center for the scapula is the first to appear. It grows in a dorso-ventral direction and gives rise dorsally to a broad supra-scapula, and ventrally fuses with the other two centers. The coracoid center is the next to appear, lying ventral to the scapula and posterior to the future procoracoid. It represents the ventral center of chondrification. The last to differentiate in the girdle formation is the procoracoid. It grows in an antero-ventral direction and forms the cephalic portion of the girdle. As soon as the centers of chondrification appear in these regions, they grow together and form a complete girdle. During the development of the girdle, the region which later gives rise to the glenoid cavity meets the opposed inward growth of the proximal epiphysis of the humerus and extends over it. Through this process of opposed growth, the head of the humerus grows in, and becomes closely applied to the girdle, forming the characteristic ball and socket joint (Figs. 23, 33). By examining successive stages during this period, one finds that the chondrification of the girdle takes place rather rapidly. Thus in a 16 mm. embryo, the girdle region is filled with undifferentiated cells, but about two days later when the embryo is 17 mm. in length, the rudiments of the three principal parts of the girdle with the glenoid cavity are already well outlined. In a slightly later stage, when the embryo is 18 mm. in length, the girdle is well along in the process of chondrification.

Following the appearances of the primordium of the girdle, the rudiments of ulna and radius differentiate respectively. At first they are two compact masses of cells with indistinct boundaries; but as soon as they are laid down, the process of chondrification proceeds rapidly. The earliest appearance of these structures is in an embryo of 17 mm. (Fig. 14), and when the embryo reaches 18 mm. in length, the ulna and radius have become definite cartilages (Fig. 16). By the time the embryo is 19 mm. long, they show the tendency to form three zones of cells along with the differentiation of diaphysis and epiphysis.

Subsequent to the differentiation of the ulna and radius, the elements in the carpal region become discernible (Figs. 15, 16). In spite of their

slightly later appearance, they are ultimately chondrified at the same time with the ulna and radius. In *Necturus*, the ulnare and intermedium, which are coalesced in later stages, arise as separate elements.

Simultaneously with the appearance of the carpal elements, four digits corresponding to the adult fingers are recognizable in the distal region of the limb (Fig. 14). They pass rapidly from the period of pre-cartilage to the stage of active chondrification when the cartilages are formed. The duration of this process of development extends from the embryo of 17 mm. to the embryo 19 mm. in length.

Owing to the rapid development of the limb skeleton beginning in embryos 16 mm. long in which centers of chondrification just become distinguishable in the different parts of the limb, and the formation of the definite structures from all rudiments in embryos 19 mm. in length, close examinations have been made in the serial sections during this period with the view of determining whether or not there occur any variations from what is found in the adult limb. So far the writer has found that as soon as all primordia become discernible, they assume the essential characteristics of the adult organs and give no indication as to their phylogenetic conditions.

2. HISTOGENESIS OF MUSCLE

A. Development of the Myoblasts

The development of the limb muscle in *Necturus maculosus* takes place at the same stage with the differentiation of the primordial skeletal centers. The first indication of its development occurs in the embryo 15 mm. in length (Fig. 10). In this stage, the blood vessels are just arising in the limb region and the undifferentiated mesenchyme shows the tendency of formation into the peripheral and the axial masses. Through the process of rapid growth of the limb bud, the distinction between these two masses of cells becomes more conspicuous in the next stage. In a transverse section of a 16 mm. embryo, the mesenchyme cells in the axial mass as shown (Fig. 11) are arranged concentrically into a condensed skeletal core, whereas the cells in the peripheral region are separated into two dorsal and two ventral masses. The dorsal masses are distributed respectively as the proximal and the distal groups of cells. The proximal group is situated on the dorso-basal region of the limb bud, while the distal group occupies the outer peripheral surface of the axial core. The ventral masses also consist of the proximal and the distal groups of cells. The proximal group is located on the ventral base of the limb just opposite the proximal group of the dorsal mass, whereas the distal group is applied to the outer ventral portion of the

limb. There is no sharp line of distinction existing between the proximal and the distal groups, but a division is indicated by the presence of a loose mass of cells between them. The regions between the dorsal and ventral masses of cells and the axial skeletal core are separated respectively by the dorsal and the ventral branches of the spinal nerve. These early differentiated masses of cells have been traced as the premuscle tissues.

In this early stage of myogenesis, the cells in the condensed premuscle masses are not arranged in any definite direction. Some of the cells are still oval in shape, but many of them are rapidly undergoing the process of elongation. The myoblasts, when they are first recognizable, possess an elongated outline, distinct nuclear membrane, and clear chromatin network consisting of many karyosomes (Fig. 19). The myoblasts increase considerably in number chiefly by mitotic division, although the transformation of the undifferentiated mesenchyme into the myoblasts also takes place in the process of growth. During the rapid differentiation of the premuscle tissues, the syncytial cytoplasm at first is indistinct, but soon appears as an extensive anastomosis among the myoblasts (Fig. 19). After the myoblasts multiply, they continue to grow with the long axes of the nuclei and cell masses in the direction of the longitudinal growth of the primordial skeletal blastema.

B. Appearance of Fibrillation

In embryos 17 mm. in length, the myofibrillae arise from the syncytial cytoplasm at first without cross striations. They appear as homogeneous structures much as the cartilage matrix appears in the early stage. Upon close examination under high magnification, two kinds of fibrillae are detected, the coarse and the fine. The coarse fibrillae are slightly spiral in outline, mixed intermittently with the fine ones which are arranged more or less in parallel lines.

As to the formation and nature of myofibrillae, many hypotheses had been held by different investigators, but the modern workers generally agree that they are intra-cellular and are formed in the cytoplasm. This view is supported by the fact that just before the appearance of the fibrillae, the syncytial cytoplasm is densely interwoven among the myoblasts (Fig. 19). As soon as the fibrillae arise, the cytoplasmic processes disappear and are replaced by the fine strands known as myofibrillae (Fig. 20). According to the observation of Carey ('20, '21), in the case of the pig, the myofibrillae arise from the cytoplasm at first as parallel rows of isolated granules which later fuse to form the delicate strands.

C. Formation of Cross Striations

The first evidence of cross striations in the limb muscles of *Necturus* is seen in the embryo 19 mm. in length (Fig. 21). In the transformation of homogeneous myofibrillae into the striated muscle, some conspicuous changes take place in the nuclei of myoblasts. The nuclei are now much longer and more pointed. The length is several times the width and they are often tapered at both ends. The karyosomes are more numerous and larger than in the preceding stages. They are arranged in an irregular axial mass and a long peripheral band. Those in the axial mass are larger than those in the periphery, while the latter are closely applied to the nuclear membrane appearing as a continuous band. It is also noticeable that the nuclei are less numerous in the striated muscle than in the preceding stage. Simultaneously with the formation of cross striations, the sarcolemma arises to invest the myofibrillae, forming a distinct muscle fiber.

3. DEVELOPMENT OF THE SPINAL NERVE

In embryos 9 mm. in length, in which the anterior limb rudiment first becomes discernible, the spinal cord as seen in transverse sections is elongated, oval in outline and with its ventro-lateral sides slightly compressed (Fig. 27). The cells in the cord are somewhat elongated and are arranged radially with respect to the curvature of the cord as a whole. They have not yet reached to the outer limiting membrane which is separated from them by a zone of clear space, in which delicate cytoplasmic processes are often found. The cell walls are indistinct with numerous large yolk granules packed together. Some of the cells at the peripheral portion remain rounded in shape. At this period, the neural crest has segregated and migrated ventrally to the middle level of the spinal cord. The nuclei in the crest are usually oval with their long axes arranged in different directions. During the downward migration of the neural crest, the sclerotome cells arise in the region between the myotome and the notochord and eventually meet those of the crest. When the cells of these two sources come into close contact with one another, their difference in origin is hardly distinguishable, since they all possess the same staining character and general outline and are heavily laden with yolk granules. Similar conditions were also observed by Harrison ('24) in the case of the frog.

When the embryo is 10 mm. in length, the cells in the spinal cord have increased greatly in size and number. They are more elongated and arranged in transverse rows, and have extended to the outer limiting membrane. Numerous mitotic figures are frequently seen. The cells in

the neural crest also have begun to elongate in the direction of their growth and have reached the ventral end of the spinal cord. The nerve fibers are beginning to appear among the neural-crest cells and run closely to the motor nerve fibers just as the latter are issuing from the ventro-lateral border of the cord.

Embryos of a slightly later stage show a definite relation between the neural crest and the motor root. The neural-crest cells condense in compact masses forming the spinal ganglia with the segmental arrangement. The cells in the ganglia which are in active mitotic division, grow downward to the middle level of the notochord (Fig. 28). As the motor fibers grow out from the cord, they pass through the spinal ganglia and fuse with their fibers. The motor fibers grow downward and outward as slender stalks to the lower part of the myotome near the ventral level of the notochord. The nerve cells found in the motor fibers are spindle-shaped, showing variable relations in the different parts of the fibers. In the intra-medullary region of the motor nerve root, where the nerve fibers take their origin and penetrate through the wall of the cord, no cells are found. As soon as the motor fibers grow out from the outer limiting membrane, they are immediately surrounded by the ganglion cells. Examination of the spinal cord shows that there is no trace of outward migration of medullary cells along the motor root at this stage. The cells in the motor root are closely arranged in the same direction with those of the spinal ganglion and appear as one stream of cells. Evidently this suggests that the migration of cells from the spinal ganglion to the motor root takes place in embryos of this period.

In embryos 13 mm. in length, the spinal ganglia become more compact (Fig. 29). The motor nerves grow further downward and outward along the median margin of the myotome as far as the somato-splanchnopleuric angle. The yolk granules have now entirely disappeared both in the spinal cord and in the spinal ganglia.

Embryos of still a little older stage show that the motor nerves have passed out beyond the lower end of the myotome and reach the base of the limb bud (Fig. 10). The nerve trunks now become more fibrous and the cells are less numerous in their distal region.

When the embryo reaches 16 mm. in length, the motor roots have entered the limb region and divide into a dorsal and a ventral branch (Fig. 11). The dorsal ramus is shorter and goes to the dorsal premuscle mass, while the ventral ramus is longer and enters the ventral premuscle mass. Enclosed between them is the dense portion of the axial skeletal blastema. The motor fibers now increase in diameter and become slightly wavy with a few spindle-shaped cells scattered in the different parts of the nerve. From this stage onward, the nerves in the limb region

are undergoing further division and union in the formation of the brachial plexus.

V. ATTAINMENT OF ADULT CONDITIONS

1. SKELETAL SYSTEM

A. Necturus 19–27 mm.

The development of the appendicular skeleton has been traced in the previous sections up to the period at which all primordia have been laid down and are rapidly undergoing the process of further differentiation. After that period, the limb skeleton continues its growth toward the adult form. The details of its development are as follows:

a. The pectoral girdle

SCAPULA (Fig. 24).—The scapula is composed of a long and slender cartilage surrounded by a layer of perichondrium. It extends dorsally to the middle level of the notochord and turns slightly mesiad as it grows downward in the curvature of the body wall. The cartilage is thin and flattened laterally. Toward both ends, the scapula increases in size.

SUPRA-SCAPULA (Fig. 25).—The supra-scapula is directly continuous with the scapula. At this period, it is composed of a condensed mass of cells which are in the process of active chondrification. It extends dorsally to the level of the ventral margin of the spinal cord. It is broader and thinner than the scapula and is surrounded by a condensed mass of mesenchyme cells.

PROCORACOID (Fig. 17).—The procoracoid arises from the ventro-anterior portion of the girdle and grows cephalad as a long cartilaginous bar. It extends from the anterior margin of the third myotome to the posterior end of the fifth. Its cephalic end is surrounded by a mass of mesenchyme.

CORACOID (Fig. 18).—The coracoid is a broad, thin piece of cartilage forming the ventral portion of the girdle. Its anterior border elongates in the direction of the procoracoid and forms a reëntrant notch in the proximal region between the junction of the procoracoid and the coracoid. The cartilage grows downward toward the ventro-median line, and is surrounded by the undifferentiated mesenchyme. At this period the coracoids have not yet met in the ventro-median line.

GLENOID CAVITY (Fig. 18).—The glenoid cavity is well outlined with distinct cartilaginous ridges around the edge. It forms a sort of cap-like

structure which encloses the head of the humerus as the latter grows into it. The wall of the cavity including the ridges is relatively thin at this period.

b. The free limb

HUMERUS (Fig. 15).—The humerus consists of a shaft and two epiphyses. The shaft is long and slender, while the epiphyses are broad. The epiphyses have surfaces which articulate with the respective neighboring cartilages. The proximal epiphysis is semi-spherical in outline with its cells arranged crowdedly into a concentric form. It presses closely against the wall of the glenoid cavity. Toward its distal end it gradually narrows down and becomes imperceptibly fused with the shaft. The distal epiphysis is larger than the proximal one, and is now beginning to differentiate into the external and internal condyles with a shallow groove between them. The whole cartilage of the humerus inclines in a postero-ventral direction.

ULNA AND RADIUS (Figs. 16, 22).—The ulna and the radius are well formed in cartilage. The olecranon process is differentiated in the proximal part of the ulna to form a sigmoid notch which fits closely over the distal epiphysis of the humerus. The middle portion of the ulna, like the shaft of the humerus, is more slender than its ends. At the distal end, its articulating surface is dorso-ventrally flattened against the ulnare and a part of the intermedium. The radius enlarges at the proximal part with its dorsal surface slightly depressed to form a distinct socket which articulates with the ball-shaped condyle of the humerus. Toward the distal end, the radius also increases in size with its surface dorso-ventrally flattened and fitted into the lateral surface of the intermedium and dorsal surface of the radiale.

CARPUS (Fig. 22).—The carpal region consists of seven cartilages arranged in three rows. The proximal row includes the ulnare, the intermedium, and the radiale. The ulnare is separated from the intermedium in the early stage, but becomes coalesced at this period leaving only a foramen for the passage of a blood vessel. The middle row consists only of a small centrale situated in the center of the carpal region. The distal row is composed of three cartilages. Carpalia 1 is lost. Carpalia 2 articulates chiefly with the outer proximal surface of metacarpal II. Carpalia 3 covers the proximal surface of the metacarpal III and also extends toward the inner proximal surface of metacarpal II. Carpaes 4 and 5 are fused to form one cartilage which articulates with the proximal ends of metacarpals IV and V.

DIGITS (Fig. 22).—The digits are four in number, the first one being

lost. The segmentation in the digits is first seen in the embryo 18 mm. in length. They are separated into distinct phalanges in embryos of 19 mm. There are nine phalanges in the digits with three in the fourth digit and two in each of the others.

There are no joint cavities between the cartilages in the distal part of the hand at this period, but each cartilage is distinctly demarcated by its deeply stained peripheral matrix.

B. Necturus 30-39 mm.

The appendicular skeleton of the larva at this period has become entirely cartilaginous and has practically attained the conditions which are comparable with the adult state, except that the scapula of the girdle and the long cartilages of the free limb have not yet become ossified. One must keep in mind, however, that even in the adult, a large proportion of the entire skeletal system remains an unossified cartilage.

a. The pectoral girdle

SCAPULA (Fig. 31).—The scapula increases greatly in size and extends farther dorsally than in the preceding stage. It becomes flattened laterally and grows broad antero-posteriorly. Its peripheral surface, as seen in transverse sections, does not contain any undifferentiated condensed mesenchyme. It is lined by a layer of thick cartilage matrix which stains dark blue with picro-indigo-carmin at this period. The cells in the cartilage are usually polyhedral in outline, with rounded nuclei, large lacunae, and heavily thickened capsules.

SUPRA-SCAPULA (Fig. 3).—The dorsal surface of the scapula broadens out into a hatchet-shaped cartilage known as the supra-scapula. It extends dorsally as far as the transverse processes of the vertebrae. There is no distinct division between the scapula and the supra-scapula in the larval stage, but they are clearly distinguishable in the adult where the scapula becomes ossified, and the supra-scapula remains cartilaginous throughout life. In transverse sections, the supra-scapula and the scapula are seen to cover almost the full extent of the lateral body wall.

PROCORACOID (Fig. 33).—The procoracoid can be studied best from the frontal sections where the full extent of the structure can easily be seen. It is the longest cartilage in the girdle. Its anterior tip extends to the level of the distal end of the fifth visceral arch. The cartilage at this period is precisely similar to the adult form. It is relatively broad at the base but immediately becomes narrow as it grows forward. The cartilage enlarges a little beyond the mid-region, taking the form of a scalpel blade with its blunt tip at the free end. The edge of the blade is directed toward the ventro-median line.

CORACOID (Fig. 34).—The coracoid is much larger than in the preceding period. It grows rapidly toward the ventro-median line and soon extends beyond it, overlapping its fellow from the opposite side. The left coracoid is usually the ventral or superficial one where the overlapping occurs. Each cartilage appears as a circular flat disc with a deep reëtrant angle near the proximal region of the procoracoid. Between the reëtrant angle and the glenoid cavity, there is a foramen through which the supra-coracoid nerve passes to supply the muscles on the ventral portion of the girdle.

GLENOID CAVITY (Fig. 32).—The glenoid cavity is on the dorsal portion of the coracoid. It is now much enlarged and surrounded with thickened cartilage which becomes elevated into a strong ridge on the external surface, forming a sufficiently deep socket for the reception of the humerus. There are also some ligamentous tissues, as seen in the adult limb, growing out from the cartilaginous ridge and becoming attached around the proximal epiphysis of the humerus. This strengthens the attachment of the free limb to the girdle.

The whole pectoral girdle now appears as a thin piece of cartilage consisting of three lobes with a cavity in the center. From this cavity, there grows out dorsally the slender scapula, which broadens out at its free end into a hatchet-shaped cartilage known as the supra-scapula. On the anterior margin of the cavity, the procoracoid grows out in an antero-ventral direction as a long, thin piece of cartilage resembling the blade of a scalpel. Below the cavity, the cartilage extends ventrally as a flat rounded plate forming the characteristic coracoid found in the urodeles.

b. The free limb

HUMERUS (Fig. 33).—The humerus has much the adult shape although it has not yet become ossified. The head is ball-shaped and fits into the glenoid cavity of the shoulder girdle. Toward the ventral surface of the head, the cartilage becomes flattened laterally and prolonged into a sharp ridge which gradually recedes toward the shaft. This ridge is known as the *crista ventralis* (Wilder, '03) and serves for the insertion of most of the ventral shoulder muscles. The humerus now is a cylindrical cartilage. It gradually enlarges toward its distal end and is imperceptibly fused with the distal epiphysis. At this period, the differentiation of the external (lateral) and the internal (median) condyles at the distal epiphysis becomes more advanced. The external condyle, which is larger and rounded in outline, fits into the depression of the head of the radius. It also serves as a point of origin for the extensor muscles of the forearm and hand. The internal condyle is less prominent and does not take part directly in the formation of the elbow

joint, though it gives origin to the flexor muscles of the forearm and hand. The groove between the two condyles articulates with the sigmoid notch and olecranon process of the ulna and also with the head of the radius.

ULNA AND RADIUS.—The ulna and radius have the same form as that found in the adult. The ulna is comparatively slender with nearly uniform thickness throughout. Its proximal end greatly elongates dorsally into an olecranon process with a prominent sigmoid notch. Its distal end becomes slightly enlarged and articulates chiefly with ulnare. The radius is slightly thicker than the ulna, and its shaft and epiphyses are quite distinct from one another. The proximal epiphysis gives rise to a sort of socket-like structure which receives the external condyle of the humerus. The distal epiphysis is laterally flattened with a slight acute apex toward the median line. It articulates with the intermedium and the radiale.

CARPUS.—The cartilages that constitute the wrist increase in size slightly and move a little apart from one another, the narrow spaces between them becoming filled with condensed cells. The arrangement of the carpal cartilages follows the same plan as that found in the adult, and is similar to the conditions described in the preceding section.

DIGITS.—The digits remain the same as in the preceding period, in regard to their number and general arrangement. Each cartilaginous segment is now differentiated into a slender shaft and has a slightly enlarged epiphysis at each end, with the exception of the terminal phalanx which has no epiphysis at the distal end.

As a whole the appendicular skeleton of *Necturus maculosus* is relatively weak in comparison with that of the higher forms, correlated with the fact that the locomotion of the animal does not depend primarily upon the limbs, but rather upon the undulating movement of the caudal fin and the entire body of the animal.

2. MUSCULAR SYSTEM

The limb muscles, as mentioned in previous sections, first arise as two dorsal (dorso-proximal and dorso-distal) and two ventral (ventro-proximal and ventro-distal) groups of condensed mesenchyme cells at the 16 mm. stage. After that stage, the premuscle masses, through processes of separation and differentiation, gradually give rise to the muscles, which grow toward the adult conditions.

A. Necturus 17.5 mm.

a. Dorso-proximal muscle mass

In embryos of this stage, several muscles have developed from this mass of premuscle, namely, *latissimus dorsi*, *dorsalis scapulae*, *anconeus scapularis*, and *anconeus coracoideus*.

LATISSIMUS DORSI (Fig. 53).—This arises as a small muscle posterior to the dorsal portion of the cartilaginous scapula. It is connected with the posterior border of the dorsalis scapulae by a mass of condensed cells. Its origin and insertion have not yet been differentiated.

DORSALIS SCAPULAE (Fig. 52).—This covers the lateral surface of the scapula extending downward from the dorsal part of the cartilage, and becomes fused at its distal end with the procoraco-humeralis near the junction between the scapula and the procoracoid.

ANCONEUS CORACOIDEUS (Fig. 53).—This arises from the posterior tuberosity of the coracoid just posterior to the glenoid cavity, and runs as far as the distal third of the humerus, where it blends with muscle fibers of anconeus scapularis.

ANCONEUS SCAPULARIS (Fig. 53).—This lies chiefly on the dorsal surface of the humerus and extends from the proximal half of the cartilage to its distal end. At this stage, there is no distinct point of insertion, since the olecranon process of the ulna has not yet become differentiated.

b. Dorso-distal muscle mass

In embryos of this stage, two masses of condensed cells are discernible on the dorsal flexor surface. One is in the antebrachial region and the other in the carpal portion. They are more or less united, and have not yet differentiated definitely into the separate extensor muscles.

c. Vento-proximal muscle mass

Several muscles have been developed from the premuscle cells of this group. They are the pectoralis, supra-coracoideus, procoraco-humeralis, coraco-brachialis longus, and humero-antebrachialis.

PECTORALIS (Fig. 52).—At this stage, this muscle arises posterior to the supra-coracoideus and partly overlaps its posterior border. It lies on the ventral side of the body posterior to the coracoid and becomes inserted upon the ventral aspect of the head of the humerus.

SUPRA-CORACOIDEUS (Fig. 52).—This appears at this stage as a broad sheet of muscle covering the ventro-proximal part of the coracoid, and follows the general outline of the cartilage. Its posterior border is overlapped by the pectoralis. It becomes inserted upon the ventral aspect of the proximal epiphysis of the humerus adjacent to the point of insertion of the pectoralis.

PROCORACO-HUMERALIS (Fig. 52).—This arises as a small mass of fibers running longitudinally with the cartilaginous procoracoid. It covers the proximal half of the ventral surface of the cartilage, and becomes

fused with the ventral end of the dorsalis scapulae at the junction between this cartilage and the scapula.

CORACO-BRACHIALIS LONGUS (Fig. 53).—This muscle lies on the median surface of the flexor side of the humerus. In a reconstruction of the limb at this stage, it is seen arising from the posterior tuberosity of the coracoid, just posterior to the glenoid cavity. It extends as far as the distal part of the humerus, where it comes into contact with a group of condensed cells, which are later to become differentiated into the flexor muscle.

HUMERO-ANTEBRACHIALIS (Fig. 52).—This muscle and the coracobrachialis longus are the two long muscles situated on the flexor side of the humerus. They correspond to the biceps of higher forms. The former lies upon the lateral side, and the latter upon the median aspect. In embryos of this stage, the humero-antebrachialis appears as a small mass of fibers covering the lateral portion of the flexor surface of the humerus. It extends from the proximal half of the cartilage to the distal end. Its origin and insertion are not clearly differentiated at this stage.

d. Ventro-distal muscle mass

At this stage, the distal part of the flexor surface of the limb is covered by condensed masses of cells which later give rise to the definite flexor muscles.

B. Necturus 19 mm.

In embryos of this stage great progress has been made in the differentiation of the muscles of the dorso-distal and ventro-distal muscle masses. The superficial layers of the extensor and the flexor muscles first become distinguishable, and the dorso-proximal and the ventro-proximal masses increase in size.

a. Dorso-proximal muscle mass

LATISSIMUS DORSI (Fig. 55).—There is no great change in this muscle at this stage. It still appears as a muscular mass growing out posterior to the dorsalis scapulae, extending dorsally and ventrally for a short distance, and ending abruptly without reaching any cartilage.

DORSALIS SCAPULAE (Fig. 54).—This increases in size and grows dorsally to the ventral margin of the rudimentary supra-scapula. It extends beyond the posterior border of the cartilage, leaving the anterior margin of the cartilage free from the muscular attachment. The distal end, as seen in the preceding stage, is fused with the procoraco-humeralis.

ANCONEUS HUMERALIS LATERALIS (Fig. 54).—This muscle lies on the lateral surface of the humerus and belongs to the same group as the anconeus scapularis and the anconeus coracoideus mentioned previously. It arises from the proximal epiphysis of the humerus, and extends obliquely along the lateral surface of the cartilage. Its fibers become fused at their distal part with the anconeus scapularis on the dorsal surface, and insert with that muscle upon the olecranon process of the ulna.

b. Dorso-distal muscle mass

Three muscles covering the extensor surface of the forearm and hand are recognizable at this stage. They are the humero-metacarpalis, extensor radialis, and the extensor ulnaris.

HUMERO-METACARPALIS (Fig. 54).—This muscle arises from the lateral condyle of the humerus and extends over almost the entire dorsal surface of the forearm to the basal margin of the digits.

EXTENSOR RADIALIS (Fig. 54).—This muscle lies on the radial side of the forearm, and entirely covers the dorsal surface of the radius. Toward the median part of the muscle, the fibers blend more or less with those of the humero-metacarpalis.

EXTENSOR ULNARIS (Fig. 54).—This muscle occupies the marginal border of the ulna and is fused dorsally with the humero-metacarpalis at this stage. It arises from the external condyle of the humerus along the margin adjacent to the olecranon process of the ulna, and becomes attached upon the lateral border of the ulna.

c. Ventro-proximal muscle mass

There is little differentiation in the muscles of this group at this stage. Besides their general growth in size, they have made no remarkable changes.

d. Ventro-distal muscle mass

The muscles that first become recognizable as the distinct flexor muscles at this stage are the palmaris superficialis, flexor ulnaris, and flexor radialis. They are more or less fused with each other.

PALMARIS SUPERFICIALIS (Fig. 55).—This is a broad mass of muscle covering the flexor surface of the antebrachium. It arises from the median condyle of the humerus between the radial and the ulnar flexors, and extends to the base of the digits.

FLEXOR ULNARIS (Fig. 55).—This muscle lies upon the flexor aspect of the entire ulna and is partly fused with palmaris superficialis. Its origin and insertion have not yet become definitely differentiated.

FLEXOR RADIALIS (Fig. 55).—This appears as a short muscle on the radial side of the palmaris superficialis, and is partly fused with the latter. It arises from the radial side of the medium condyle of the humerus, and inserts upon the outer margin of the radiale.

C. Necturus 21-39 mm.

In embryos of this period, the appendicular muscles are growing toward the conditions which are comparable with those of the adult limb. The muscles in the distal part of the limb have separated into the superficial and the deep layers. Several muscles which are not developed in the girdle and upper-arm at the preceding stages are now differentiated.

a. Dorso-proximal group of muscles

LATISSIMUS DORSI (Figs. 37, 56-59).—This muscle at this time is broad and fan-shaped, and covers the lateral side of the body between the fifth and eighth myotomes. It extends much farther dorsally than the supra-scapula, and gradually narrows down toward its ventral end where it is inserted into the anterior lateral lip of the glenoid cavity by the ligamentous fibers, as seen in the adult.

DORSALIS SCAPULAE (Figs. 37, 56-59).—During the further growth of the supra-scapula in embryos of this period, the dorsalis scapulae also increases greatly in length. It now originates near the free margin of the supra-scapula, and runs somewhat obliquely along the scapula. Hence the muscle is free from the distal half of the cartilage and converges to insert into the lateral surface of the proximal epiphysis of the humerus.

TRAPEZIUS (Figs. 37, 56-59).—This muscle appears slightly later than those mentioned above. It becomes distinguishable first in the embryo of 21 mm. At this stage, it is seen on the anterior margin of the cartilaginous scapula. It is broad at the dorsal end, but gradually becomes narrow as it grows downward toward the middle of the scapula. In embryos between 27 mm. and 39 mm., the muscle begins to grow in both the dorsal and ventral directions, as in the case of the latissimus dorsi. Dorsally it grows into a broad, thin sheet between the second and fourth myotomes, and extends beyond the dorsal margin of the supra-scapula. Ventrally it converges gradually into a narrow strip along the anterior margin of the scapula, and becomes inserted at the distal end of the cartilage. The dorsal part of the muscle is covered laterally by the levator arcum and dorso-laryngeus.

OMOHYOIDEUS (Fig. 37).—This muscle is seen best in sagittal sections. In embryos between 21 mm. and 23 mm., it blends more or less

with the visceral muscles, and can hardly be distinguished as a separate muscle. In embryos 34 mm. to 39 mm., it appears as a narrow muscle situated antero-ventrally to the trapezius. Its fibers arise from the third epibranchial arch, and run obliquely from an antero-dorsal to a postero-ventral direction, inserting into the deep notch between the scapula and the procoracoid. Its dorsal part is overlapped laterally by the dorso-laryngeus.

LEVATOR ANGULI SCAPULAE (Figs. 38, 56-58).—The first appearance of this muscle occurs in the embryo 21 mm. in length. At this stage, it is a short, narrow muscle arising anteriorly to the supra-scapula, and becoming inserted at its anterior margin. In embryos 27 mm. to 39 mm., it increases greatly in length, and appears as a long narrow band extending horizontally between the third epibranchial arch and the anterior margin of the supra-scapula. It is covered laterally by the levator arcuum, dorso-laryngeus, and trapezius. Its point of insertion is at the inner margin of the anterior border of the supra-scapula. In the adult, the muscle arises from the posterior end of the exoccipital as a fine tendon which broadens into fleshy fibers toward the supra-scapula. This tendinous tissue is not yet differentiated in the larval stage.

SERRATUS ANTERIOR (Figs. 38, 56-58).—In the embryo 21 mm. in length, this muscle is composed of a small mass of fibers lying on the median side of the supra-scapula, and extending from the fifth to the seventh myotomes. It is entirely covered laterally by the latissimus dorsi. When the embryo reaches 27 mm. in length, the muscle increases in length posteriorly, and also extends farther anteriorly along the inner surface of the supra-scapula to the region near to the insertion of the levator anguli scapulae. The muscle arises as a narrow strip posterior to the supra-scapula and gradually broadens and separates into several strips near its insertion upon the median surface of the cartilage.

SCAPULO-HUMERALIS (Figs. 36, 57).—This muscle first arises in the embryo 21 mm. in length as a narrow band of muscle extending from the deep sinus between the scapula and the tuberosity of the coracoid to the proximal part of the humeral shaft. In the 27 mm. embryo, the muscle enlarges near its point of origin, and runs distally between anconeus scapularis and anconeus coracoideus toward the humerus. It inserts upon the inner surface of the mid-region of the cartilaginous shaft.

ANCONAEUS SCAPULARIS (Figs. 35, 57, 58).—This is a large muscle covering the dorsal surface of the humerus. It arises from the lateral edge of the glenoid cavity as ligamentous fibers which are continuous with the insertion of latissimus dorsi. Thus the origin of the former appears to be the insertion of the latter.

ANCONEUS HUMERALIS LATERALIS (Fig. 35).—This is a narrow band of muscle running obliquely along the lateral surface of the humerus. It arises from the proximal end of the cartilaginous shaft, and extends obliquely toward the distal end of the cartilage. It blends with the anconeus scapularis at the insertion upon the olecranon process.

ANCONEUS CORACOIDEUS (Figs. 35, 56-58).—This muscle covers the median surface of the humerus, and extends somewhat obliquely from the posterior tuberosity of the coracoid to the distal half of the humerus, where it fuses with the median edge of the anconeus scapularis.

ANCONEUS HUMERALIS MEDIALIS (Fig. 35).—This is a deep muscle closely applied to the entire dorsal surface of the shaft of the humerus. It is overlapped by the anconeus scapularis and anconeus humeralis lateralis, but is separated from them by large blood vessels which pass between them.

b. Dorso-distal group of muscles

HUMERO-METACARPALIS (Fig. 42).—In the embryo of 21 mm., this muscle appears as a large triangular mass. Toward the distal end, it separates into three strips which end in the interdigital spaces between the metacarpals.

EXTENSOR RADIALIS SUPERFICIALIS (Fig. 41).—This is the superficial strip of the extensor radialis. It arises from the external condyle of the humerus, and lies along the free margin of the radius. The deep fibers of this muscle become attached to the radius in their course to the insertion upon the radiale and carpalia 2. The muscle can be seen superficially from the lateral aspect of the radius.

EXTENSOR ULNARIS (Fig. 41).—At this period, this appears as a distinct, separate muscle, although the general outline remains the same as in the preceding stage.

EXTENSOR RADIALIS PROFUNDUS (Fig. 41).—This muscle is covered by the humero-metacarpalis and is situated medial to the extensor radialis superficialis. Its point of origin is at the ventral end of the external condyle, being distal to that of the superficialis. The muscle is inserted along the ulnar aspect of the radius.

SUPINATOR (Fig. 41).—This is a short muscle running obliquely in the carpal region and is first seen in the embryo of 22 mm. A great part of this muscle is covered superficially by the humero-metacarpalis, and only its distal portion can be seen from the radial margin of the latter muscle. It arises from the radial side of the ulnare-intermedium and extends obliquely to the lateral side of the proximal part of metacarpal II.

EXTENSOR BREVIS (Fig. 60).—This consists of four narrow strips of muscle which arise from the distal margins of the carpales, becoming distinguishable in the embryo 21 mm. in length. The first and second strips have their points of origin at the carpales 2 and 3, and the third and fourth at the carpalia 4 + 5. They lie along the dorso-median surface of the digits and insert upon the bases of the terminal phalanges.

c. Vento-proximal group of muscles

PECTORALIS (Figs. 39, 57-59).—In the early stages (17.5-19 mm.) this muscle arises posteriorly to the supra-coracoideus and partly overlaps its posterior end. The two are somewhat connected by a mass of condensed cells. In embryos between 27 mm. and 39 mm., the muscle extends further postero-ventrally toward the linea alba, and becomes a large sheet of muscle covering an extensive area between the coracoid and the ninth myotome. Its anterior part is still closely applied to the posterior part of the supra-coracoideus. These two muscles run in the same direction, and can be mistaken readily for a continuous piece of muscle. In transverse sections through the pectoral region, it is seen that the pectoralis on each side of the body grows rapidly toward the ventro-median line, and finally comes to lie beside the linea alba. In frontal sections, the fibers of the pectoralis are shown to be growing in various directions. The most anterior fibers at the level of the mid-region of the coracoid, incline slightly postero-laterally. Those just behind the posterior margin of the coracoid are arranged at right angles to the linea alba, while those in the middle part of the muscle run obliquely. Toward the distal part of the muscle, the fibers lie nearly longitudinal. From their extensive origin along the linea alba, the muscle fibers converge toward the region between the proximal part of the humerus and the coracoid, and finally insert upon the crista ventralis of the humerus.

SUPRA-CORACOIDEUS (Figs. 34, 39).—The general outline of this muscle remains the same as that in the preceding stages. Its insertion becomes clearly differentiated upon the crista ventralis just proximal to the insertion of the pectoralis.

PROCORACO-HUMERALIS (Figs. 33, 59).—During this period, the muscle increases both in length and width. It grows further anteriorly, and extends from the lateral edge to the median edge of the procoracoid. It is inserted upon the crista ventralis of the humerus. While this muscle is growing, the rectus superficialis hypobranchialis posterior also becomes differentiated from the trunk muscles. The latter has an extensive origin anterior to the procoracoid, and grows posteriorly over its free end, becoming fused with the anterior part of the procoraco-humeralis on the dorso-lateral side of the cartilage.

RECTUS SUPERFICIALIS HYPOBRANCHIALIS POSTERIOR (Figs. 57-60).—This muscle becomes distinguishable in the embryo 21 mm. in length. At that stage, the muscle appears as a single strip arising anterior to the free end of the procoracoid. It begins to separate into the external (lateral) and the internal (median) strips as it enters the cartilage. The external strip is partly fused with the procoraco-humeralis on the lateral side of the procoracoid. The internal strip is closely applied to the distal part of the median surface of the cartilage. These two strips are partially connected with each other on the dorsal edge of the cartilage between the lateral and the median surfaces. In embryos between 34 mm. and 39 mm., the separation between the lateral and the median strips becomes more distinct. They originate as a single band of muscle from the posterior end of the first ceratobranchial arch, but soon separate as they grow posteriorly toward the cartilaginous procoracoid. The median strip now arises from the anterior end of the cartilage, and grows along its median margin for a long distance, finally converging into a narrow insertion upon the external surface of the girdle just anterior to the glenoid cavity. The lateral strip courses along the lateral side of the procoracoid and partly fuses with the procoraco-humeralis as it inserts into the notch between the distal end of the scapula and the proximal base of the procoracoid.

CORACO-BRACHIALIS LONGUS (Figs. 37, 56-58).—This muscle now becomes separated from the anconeus coracoideus. It arises from the posterior tuberosity of the coracoid and inserts upon the distal half of the shaft of the humerus.

CORACO-BRACHIALIS BREVIS (Figs. 36, 37).—This is a small, deep muscle covered medially by the coraco-brachialis longus, and laterally by the supra-coracoideus. It first becomes discernible in the embryo 21 mm. in length, and attains the adult condition at 27 mm. It arises from the posterior margin of the coracoid just ventral to the tuberosity, and extends slightly dorsally along the median aspect of the humerus, becoming inserted upon the proximal half of the cartilaginous shaft.

d. Ventro-distal group of muscles

PALMARIS SUPERFICIALIS (FIG. 56-58).—This muscle increases greatly in size, and extends distally to insert into the proximal margin of the palmar fascia. The latter arises as an aponeurotic sheet from the distal margin of the palmaris superficialis and reaches to the base of the metacarpals, where it separates into four tendon-like strips. They lie along the palmar aspect of the four digits, and finally become inserted upon the bases of the terminal phalanges.

FLEXOR ULNARIS (Fig. 40).—This now becomes a distinct muscle, and lies upon the entire flexor aspect of the ulna. It originates from the median condyle of the humerus adjacent to the olecranon process, and extends distally along the outer margin of the ulna. The fibers converge into a narrow insertion upon the lateral aspect of the ulnare-intermedium and the carpalia 4 + 5.

FLEXOR RADIALIS (Figs. 40, 56-58).—The general outline remains the same as in the preceding period. This appears as a distinct separate muscle without any fusion with the palmaris superficialis.

PALMARIS PROFUNDUS (Fig. 40).—This belongs to the deep series of muscles, and is covered externally by the palmar fascia. It arises from the radio-flexor aspect of the ulna, and runs across the surface of ulnare-intermedium. It is attached to the inner surface of the palmar fascia.

ULNARICARPALIS (Fig. 40).—This is first seen in the embryo of 21 mm. It is a narrow muscle situated between the palmaris profundus and the pronator. The fibers run longitudinally from the radial aspect of the ulna, extending downward to carpalia 4 + 5.

PRONATOR (Fig. 40).—This muscle is directly opposite the supinator on the extensor surface. The fibers run obliquely from the middle of the shaft of the ulna to the radial margin of the base of metacarpal II. The distal part of the muscle can be seen from the radial side of the palmar fascia.

CARPO-METACARPALIS (Fig. 41).—This consists of a group of deep muscles extending from the carpus to the metacarpals. They arise from the distal margins of carpals and insert into the distal end of the metacarpals. Two strips of the muscle are found on each metacarpal, one of which lies on the radial side, and the other on the ulnar side. In digit II, both the radial and ulnar strips arise from the distal margin of carpalia 3 and become attached to the ulnar border of metacarpal II. In digit III, the radial strip arises from carpalia 3, and the ulnar from the interspace between carpalia 3 and carpalia 4 + 5. In digit IV, both the radial and the ulnar strips originate from carpalia 4 + 5. In digit V, the radial and the ulnar fibers are larger than those found in the other digits. The radial strip arises from the ulnar half of the margins of carpalia 4 + 5 and inserts upon the radial margin of metacarpal V, while the ulnar strip grows out chiefly from the ulnar side of the distal margin of the ulnare-intermedium, and becomes attached to the ulnar margin of metacarpal V.

INTERMETACARPALES (Fig. 42).—There are three of these small muscles in the interdigital spaces in the deep region of the hand. These muscles appear somewhat triangular in shape and extend somewhat obliquely between the metacarpal cartilages.

In order to avoid confusion in using the terminology of the pectoral musculature of *Necturus*, a list of synonyms of the pectoral muscles is given here:

<i>Mivart</i> ('69)	<i>Wilder</i> ('12)	<i>Adams</i> ('26)
Latissimus dorsi	Latissimus dorsi	Latissimus dorsi
Deltoid	Dorsalis scapulae	Dorsalis scapulae
Trapezius	Trapezius	Cucullaris
Omohyoid	Omohyoideus	Omohyoid
Levator anguli scapulae	Levator anguli scapulae	Levator scapulae
Serratus magnus	Serratus anterior	Serratus magnus
Pectoralis	Pectoralis	Pectoralis
Subclavius	Procora-humeralis	Procoraco- humeralis
Subclavius ? (Part of above)	Rectus superficialis hypobranchialis posterior	Rectus superfic- ialis hypobranchialis posterior
Triceps	Anconeus	Triceps (Quadriceps)
Biceps	Humeroantibrachialis	Biceps
Coraco-brachialis (1st strip)	Supracoracoideus	Supracoracoid
Coraco-brachialis (2nd strip)	Coraco-brachialis longus	Coraco-brachialis
.....	Coraco-brachialis brevis	Coraco-brachi- alis brevis
Subscapularis	Scapulo-humeralis	Scapulo-humeralis
Extensor longus	Humerometacarpalis	Extensor metacarpalis
Supinator longus	Extensor radialis superficialis	Extensor radialis
Ulnaris	Extensor ulnaris	Extensor ulnaris
Extensor brevis	Supinator	Supinator
.....	Extensores breves
Pronator quadratus ?	Extensor radialis profundus
Flexor longus	Palmaris superfic- ialis	Palmaris superficialis
Pronator teres (2nd strip) ?	Palmaris profundus
Flexor brevis	Pronator	Pronator
.....	Ulnaricarpalis	Ulnaricarpalis
Pronator teres (1st strip)	Flexor ulnaris	Flexor ulnaris
.....	Flexor radialis	Flexor radialis
.....	Carpo-metacarpales
.....	Intermetacarpales

3. BRACHIAL PLEXUS

In order to get a clear picture of the brachial plexus in *Necturus maculosus* during the developmental period, a wax reconstruction of the brachial plexus in a larva of 34 mm. in length was made (Figs. 61-63). The plexus has attained the adult form at this stage. The brachial plexus is formed from the branches of the ventral roots of the spinal

nerves III, IV, and V, the spinal nerve IV forming the chief trunk (Fig. 30). The details of these nerves are as follows:

SPINAL NERVE III.—As soon as this nerve comes out from the spinal cord, it grows posteriorly toward the limb region. Before it enters the plexus, it separates into three branches. The anterior branch is small and is distributed to the body wall anterior to the girdle. The middle one is long and slender. It passes through the foramen between the glenoid cavity and the reëtrant angle of the coracoid forming the supra-coracoid nerve. It innervates the muscles on the ventral portion of the girdle. The posterior branch is slightly larger than either of the first two. It joins the spinal nerve IV forming the plexus.

SPINAL NERVE IV.—This is the largest branch of the plexus, both in the larva and in the adult, and runs along the posterior margin of the scapula. At the distal part of this cartilage, the nerve anastomoses with the posterior ramus of the spinal nerve III forming the plexus.

SPINAL NERVE V.—This is the most posterior nerve contributing to the formation of the brachial plexus. It goes anteriorly, and just before reaching the plexus, it gives rise to two rami: the anterior ramus joins the spinal nerve IV in the plexus, while the posterior one is distributed to the body wall posterior to the girdle.

VI. EXTERNAL DEVELOPMENT OF THE LIMB

The foregoing descriptions are confined to the development of the internal structures of the anterior limb. A brief account concerning the development of the external form of the appendage may be given as follows:

The first indication of the external appearance of the limb bud is in an embryo 13 mm. in length. At this stage, the bud projects dorsally (Fig. 43) and measures about .5 mm. in length. In a slightly later stage, when the embryo is 15 mm. long (Fig. 44), the limb bud increases in size and measures about .8 mm. in length. It now inclines in a dorso-posterior direction at an angle of 45° to the long axis of the embryo. When the embryo reaches 16 mm. in length, the inclination of the limb bud increases to 75° (Fig. 45). It still bears no indication of separation into digits.

The first evidence of the digits occurs when the embryo is 17 mm. in length (Fig. 46). At this stage, the limb bud grows in a postero-ventral direction and has three digits. These are the second, third, and fourth fingers. In *Necturus*, the first one is lost and does not reappear during the ontogenetic development. From the examination of sections, it is

found that the rudiments of all four fingers (2nd, 3rd, 4th, and 5th) appear at the same stage, although their external indications are not shown simultaneously.

In an embryo of 18 mm., the limb bends and grows farther ventrally (Fig. 47). The convex aspect of the bending is directed dorsally, and the concave aspect toward the ventral side. At this stage also, the fifth finger becomes visible. Soon after the bending takes place in the limb, the division between the upper arm and the antebrachial region begins to become distinguishable. This division is noticeable in an embryo 19 mm. in length (Fig. 48). At this stage, the four fingers are stout and have blunt tips.

In the subsequent stages (20-33 mm.), the free limb increases in size and gradually assumes the adult features (Figs. 49-51). The upper arm grows nearly longitudinally to the long axis of the body, while the forearm and hand develop in a postero-ventral direction at an angle of about 45° to the upper arm.

VII. DISCUSSION

1. FATE OF THE MYOTOME PROCESSES

Considerable work has been done on the relation of the myotomes to the early development of the limb in vertebrates. The results obtained, however, vary with the different investigators. Thus far there are two views regarding the fate of the myotome processes: (1) that the myotomes or their processes take part in the formation of the limb; (2) that the myotomes are in no way concerned in the formation of the limb, which arises as a thickening of the somatopleure.

According to Goette ('75), the limb muscles in *Bombinator* develop from the outer layer of the muscle plate. Jordan ('88), in his paper on the development of the anterior limb in *Anura*, accepts the viewpoint set forth by Goette. Kaestner ('93) believes that the myotomes take part in the formation of the limb in *Anura* at a very early stage. Field ('94) also described the limb muscles as being derived from the myotome processes in both urodeles and anurans. Mollier ('95) observed the growth of the muscle buds from the myotomes to the limb bud and their transformation into the limb muscles in *Lacertilia*. Later Tschernoff ('07) through his study on the development of the hind limb in the frog, concluded that the primordium of the limb is formed partly from the cells proliferated from the myotomes and partly from those derived from the somatopleure.

On the other hand, Paterson ('88), in his work on the fate of the muscle plate in the chick, thought that the muscle plate does not pass into the limb region, but is later transformed into the longitudinal muscles of the trunk. The limb muscles, he thought, are formed by the differentiation of the mesenchyme cells which form the primitive limb bud. Harrison ('95), in his study on the development of fins in teleosts, found that the ventral myotome processes in the region of the pectoral fin take no part in the formation of the muscles in the fin, but are differentiated into the coraco-hyoid muscle. The structures of the pectoral fin, including the cartilages and muscles, according to Harrison, are differentiated from the mesenchyme cells which develop from the somatopleure. Similar conditions were also described in his later work ('18), on the development of the fore limb in *Amblystoma*, in which he found that the limb develops as a thickening of the somatopleure in the region between the third and fifth myotomes. Byrnes ('98), through both her observation and experimental work on the development of the limb in urodeles and anurans, confirmed the results obtained by Harrison, and concluded that the ventral myotome processes in *Amphibia* give rise to the ventral muscles of the body wall, and take no part in the formation of the limb. Lewis ('10) proved by his experimental work that the limb in *Amblystoma* can develop normally in the absence of the myotomes in the limb region. His results were later confirmed by Detwiler ('18).

On the basis of the evidence of the present work on *Necturus maculosus*, the writer agrees with the viewpoint set forth by the second group of investigators that the limb arises as a thickening of the somatopleure, and is in no way derived from the myotomes or their processes. This conclusion is justified on the following grounds:

- (1) The primordial anterior limb bud is found as a thickening of the somatopleure before the appearance of the ventral myotome processes in an embryo of 9 mm.

- (2) The proximity of the limb bud and the myotome processes is merely due to the excessive growth of the pronephric tubules, which press the processes closely against the median side of the limb bud.

- (3) During their close contact with the limb bud, the myotome processes are distinguished from the surrounding mesenchyme cells by their dorso-ventrally elongated nuclei and by their well-defined general form. No indication of a migration of cells from the myotome processes to the limb bud has been found.

- (4) When the ventral processes are cut off from their respective myotomes, they grow downward along the outer border of the coelom, and later transform definitely into the ventral muscles of the body wall.

2. FORMATION OF THE LIMB SKELETON

The early development of the limb skeleton in *Necturus* is quite similar to that of higher forms. Carey ('21), in his work on the genesis of bone and muscle in the pig, found that the primordial skeletal center in the limb bud arises by condensation of the undifferentiated mesenchyme cells. He also observed that from this center of condensation, the growth of the limb skeleton takes place in a proximo-distal direction. In *Necturus*, similar conditions also occur in the formation of the limb skeleton. Soon after the mesenchyme cells in the limb bud divide into the peripheral and the axial masses in the 16 mm. embryo, the chondrification of the cells in the axial mass immediately takes place. This is followed first proximally by the appearance of the rudiments of the girdle, and then distally at a slightly later stage by the differentiation of the skeletal centers of the free limb.

In the differentiation of the pectoral girdle, three centers of chondrification are distinguishable. The center for the scapula is the first to appear, and is followed by the center for the coracoid and finally by the procoracoid center. The suprascapula has no special center of chondrification, but appears as a direct continuous structure of the scapula in the larval stage. This manner of chondrification of the pectoral girdle of *Necturus* is closely in accord with the observations made by Wiederheim ('89) in *Triton*, *Salamandra*, and *Siredon*, and Detwiler ('18) in *Amblystoma*.

As to the structure of the matrix of the cartilage, most of the early workers regarded this ground substance as a simple, homogeneous material. However, by using picro-indigo-carmin stain, the matrix stains deep blue, and appears, under high magnification, as a spongy network of fine fibers. The presence of the fibers in the matrix has been observed also by Fell ('25) in the development of the cartilage in the chick. Thus the view held by the early workers regarding the matrix as a simple and homogeneous substance now becomes untenable.

3. DIFFERENTIATION OF THE LIMB MUSCLES

Paterson ('88) observed that the limb muscles in the chick arise at first as the dorsal and ventral masses of condensed mesenchyme cells, between which the primordial skeletal center lies. Romer ('27) in his work on the development of the thigh musculature described the appendicular muscles as developing from these opposed dorsal and ventral pre-muscle masses. On the basis of his comparative studies in the appendicular musculature of the adult primitive tetrapods ('22, '24), and his work on the development of the thigh musculature in the chick, he concluded

that the limb muscles of higher tetrapods are homologous with the two opposed masses of muscles in the paired fins of fish, and are derived from them.

Ontogenetic evidence in the present work on *Necturus* is similar to that of Romer's work on the chick. The limb muscles in *Necturus* first arise as the dorsal and ventral premuscle masses. Each of these separates at the elbow region into the proximal and the distal portions. From these premuscle masses the adult limb muscles are gradually developed. In the chick the dorsal and ventral premuscle masses also separate into the proximal and the distal portions at the knee region. The embryological facts obtained from the present investigation add further support to the theory first advanced by Romer that the limb musculature of tetrapods originated from the two opposed muscle masses of the fish fin.

The mode of the differentiation of the pectoral musculature of *Necturus* is summarized in the accompanying tables.

4. DEVELOPMENT OF THE LIMB PLEXUS

Paterson ('88) found that there are four steps in the process of the development of the limb plexus in birds and mammals. He says: "In the development of the nerves in the limbs the following steps occur. The primitive nerve, in the first place, grows out beyond the lower end of the muscle plate, and reaches the root of the limb. It there, secondly, spreads out into an irregular series of processes, which pass into the undifferentiated tissue of the limb. Thirdly, these branches at a later date arrange themselves in two trunks, one dorsal, the other ventral, which extend still farther into the limb and enclose between them a mass of blastema, from which the cartilaginous basis of the limb is formed. Fourthly, the dorsal and ventral trunks fuse with adjacent dorsal and ventral trunks to form two broad flat bands, from which still later, the individual nerves as found in the adult are produced."

The development of the brachial plexus in *Necturus* is primarily similar to the conditions described by Paterson in the case of birds and mammals, except that the third step in the process does not occur in this form. As the spinal nerves grow out from the spinal cord at the 10 mm. stage, they fuse with the ganglion fibers, and extend downward along the median margins of the myotomes. When the embryo reaches 14 mm. in length, they pass out beyond the lower ends of the myotomes to the base of the limb bud. At the 16 mm. stage, the spinal nerves give rise directly to the dorsal and the ventral branches which supply the dorsal and the ventral masses of primitive muscle, without separating into several irregular branches. In the later stage of the development of the spinal nerves, as shown in the reconstruction of the brachial plexus of a 34 mm.

TABLE I

Muscles	First appearance	Origin	Insertion
<i>Dorso-proximal group</i>			
<i>Latissimus dorsi</i>	17.5 mm.	between 5th and 8th myotomes	antero-lateral edge of glenoid cavity
<i>Dorsalis scapulae</i>	17.5 mm.	lateral aspect of supra-scapula	lateral surface of proximal humeral epiphysis
<i>Trapezius</i>	21 mm.	between 2nd and 4th myotomes	anterior border of scapula
<i>Omohyoideus</i>	34 mm.	3rd epibranchial arch	notch between scapula and coracoid
<i>Levator anguli scapulae</i>	21 mm.	posterior end of exoccipital	antero-medial margin of supra-scapula
<i>Serratus anterior</i>	21 mm.	between 5th and 7th myotomes	postero-medial margin of supra-scapula
<i>Scapulo-humeralis</i>	21 mm.	margin between scapula and coracoid tuberosity	median mid-region of shaft of humerus
<i>Anconeus scapularis</i>	17.5 mm.	antero-lateral edge of glenoid cavity	olecranon process of ulna
<i>Anconeus coracoideus</i>	17.5 mm.	posterior tuberosity of coracoid	olecranon process of ulna
<i>Anconeus humeralis lateralis</i>	19 mm.	lateral surface of shaft of humerus	olecranon process of ulna
<i>Anconeus humeralis medialis</i>	34 mm.	dorsal surface of shaft of humerus	olecranon process of ulna
<i>Dorso-distal group</i>			
<i>Humero-metacarpalis</i>	19 mm.	lateral condyle of humerus	bases of metacarpals
<i>Extensor radialis superficialis</i>	19 mm.	lateral condyle of humerus	radiale and carpalia 2
<i>Extensor radialis profundus</i>	21 mm.	lateral condyle of humerus	ulnar aspect of radius
<i>Supinator</i>	21 mm.	ulnare-intermedium	lateral base of metacarpal II
<i>Extensor ulnaris</i>	19 mm.	lateral condyle of humerus	lateral margin of ulnare
<i>Extensores breves</i>	21 mm.	distal margins of carpales	bases of terminal phalanges

TABLE II

Muscles	First appearance	Origin	Insertion
<i>Ventro-proximal group</i>			
Pectoralis	17.5 mm.	9th myotome distal, lateral part of coracoid first ceratobranchial arch	crista ventralis of humerus
Supra-coracoideus	17.5 mm.		crista ventralis of humerus
Rectus superficialis hypobranchialis posterior ..	21 mm.		2 insertions: (a) notch between scapula and procoracoid; (b) anterior to glenoid cavity
Procoraco-humeralis	17.5 mm.	antero-lateral half of procoracoid	crista ventralis of humerus
Coraco-brachialis longus	17.5 mm.	posterior tuberosity of coracoid	distal half of humeral shaft
Coraco-brachialis brevis	21 mm.	posterior margin of coracoid	proximal half of humeral shaft
Humero-antibrachialis	17.5 mm.	crista ventralis of humerus	radial edge of radius
<i>Ventro-distal group</i>			
Palmaris superficialis	19 mm.	median condyle of humerus	proximal margin of palmar fascia
Palmaris profundus	21 mm.	radio-flexor aspect of ulna	inner (median) surface of palmar fascia
Ulnarcarpalis	21 mm.	radial aspect of ulna	carpalis 4+5
Flexor radialis	19 mm.	radial aspect of median condyle of humerus	lateral margin of radiale
Flexor ulnaris	19 mm.	ulnar aspect median condyle of humerus	ulnare-intermedium and carpalia 4+5
Pronator	21 mm.	radial side of ulnar shaft	radio-basal region of metacarpal II
Carpometacarpales	21 mm.	distal margins of carpales	shafts of metacarpals
Inter-metacarpales	21 mm.	radial aspect of metacarpals III to V	ulnar margins of metacarpals II to IV

larva, the dorsal and the ventral branches of the spinal nerves III, IV, and V have combined to form the plexus, which resembles that of the adult.

VIII. CONCLUSIONS

1. The primordial limb bud in *Necturus maculosus* arises as a thickening of the somatopleure before the appearance of the ventral myotome processes in the embryo of 9 mm.

2. The ventral myotome processes appear in the 10 mm. embryo and grow downward toward the limb region. At the 13 mm. stage, they are cut off from their respective myotomes and are pressed closely against the base of the limb by the excessive growth of the pronephric tubules. Although the ventral myotome processes lie in close contact with the mesenchyme cells in the limb bud, there is no indication of a migration of cells from the processes to the limb bud. The isolated myotome processes later migrate downward along the outer border of the coelom, and transform finally into the muscles of the ventral body wall in the embryo of 17 mm.

3. The first appearance of the primordial center of chondrification occurs in the 16 mm. embryo as a condensed mass of mesenchyme cells in the center of the limb bud. From this center of chondrification, the growth of the limb skeleton takes place in a proximo-distal direction. Proximally, the blastema of the pectoral girdle soon becomes distinguishable, and distally at a slightly later stage, the rudiments of the free limb are differentiated. In an embryo of 18 mm., all primordia of the limb skeleton are discernible.

4. The anterior limb skeleton in larvae between 30 mm. and 39 mm. has practically attained the conditions comparable with that in the adult stage, except that the scapula of the girdle and the long cartilages of the free limb have not yet become ossified.

5. The limb muscles arise first as dorsal and ventral masses of condensed mesenchyme cells in the embryo of 16 mm. At the 17.5 mm. stage, the limb muscles first begin to differentiate definitely from these opposed premuscle masses. In larvae from 21 to 39 mm. in length, the appendicular musculature has attained conditions similar to those of the adults. The mode of development suggests that the limb musculature of tetrapods originated from the opposed muscle masses of the fish fin.

6. The brachial plexus of *Necturus* is formed from the ventral roots of the spinal nerves III, IV, and V. The motor nerves first appear in the embryo of 10 mm., growing downward along the medial margins of the myotomes, and reaching the base of the limb bud in the 14 mm. embryo.

When the spinal nerves enter the limb region at the 16 mm. stage, they divide into the dorsal and the ventral branches. These branches grow together in the formation of the brachial plexus by the time the embryo reaches 34 mm. in length.

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EXPLANATION OF PLATES

ABBREVIATIONS

<i>ac</i> anconeus coracoideus	<i>ld</i> latissimus dorsi
<i>ahl</i> anconeus humeralis lateralis	<i>mp</i> myotome process
<i>ahm</i> anconeus humeralis medialis	<i>mtc</i> metacarpal
<i>as</i> anconeus scapularis	<i>my</i> myotome
<i>caf</i> carpal foramen	<i>nv</i> nerve
<i>cbb</i> coracobrachialis brevis	<i>om</i> omohyoideus
<i>cbl</i> coracobrachialis longus	<i>op</i> olecranon
<i>ce</i> centrale	<i>p</i> pronator
<i>cm</i> carpometacarpales	<i>pc</i> procoracoid
<i>co</i> coracoid	<i>pch</i> procoracohumeralis
<i>cp</i> carpalia	<i>pe</i> pectoralis
<i>cru</i> crista ventralis	<i>pf</i> palmar fascia
<i>de</i> distal epiphysis	<i>pp</i> palmaris profundus
<i>dig</i> digits	<i>ps</i> palmaris superficialis
<i>ds</i> dorsalis scapulae	<i>pt</i> pronephric tubules
<i>eb</i> extensor brevis	<i>r</i> radius
<i>er</i> extensor radialis	<i>ra</i> radiale
<i>erp</i> extensor radialis profundus	<i>rshp</i> rectus superficialis hypobranchialis posterior
<i>ers</i> extensor radialis superficialis	<i>s</i> scapula
<i>eu</i> extensor ulnaris	<i>sa</i> serratus anterior
<i>fr</i> flexor radialis	<i>sc</i> supra-coracoideus
<i>fu</i> flexor ulnaris	<i>sh</i> scapulo-humeralis
<i>gc</i> glenoid cavity	<i>spg</i> spinal ganglion
<i>gd</i> girdle	<i>spn</i> spinal nerve
<i>h</i> humerus	<i>ss</i> supra-scapula
<i>ha</i> humeroantibrachialis	<i>su</i> supinator
<i>hm</i> humerometacarpalis	<i>tp</i> trapezius
<i>im</i> intermedium	<i>u</i> ulna
<i>imt</i> intermetacarpales	<i>ua</i> ulnare
<i>las</i> levator anguli scapulae	<i>uc</i> ulnaricarpalis
<i>lb</i> limb bud	<i>ui</i> ulnare-intermedium
	<i>y</i> yolk

PLATE I

FIG. 1.—Transverse section of a 9 mm. embryo showing the primordial limb bud before the appearance of the ventral myotome process. $\times 47$

FIG. 2.—Transverse section of a 10 mm. embryo showing the beginning of the ventral myotome process in the limb region. $\times 47$

FIG. 3.—Transverse section of an 11 mm. embryo showing the ventral myotome process growing over the pronephric tubules. $\times 47$

FIG. 4.—Transverse section of a 12 mm. embryo showing the migration of the ventral myotome process to the ventral base of the limb bud. $\times 47$

FIG. 5.—Frontal section of a 13 mm. embryo showing the position of the limb bud between the third and fifth myotomes. $\times 19$

FIG. 6.—Enlarged view of the above limb bud. $\times 47$

FIG. 7.—A group of primitive mesenchyme cells in the limb bud of a 9 mm. embryo. $\times 350$

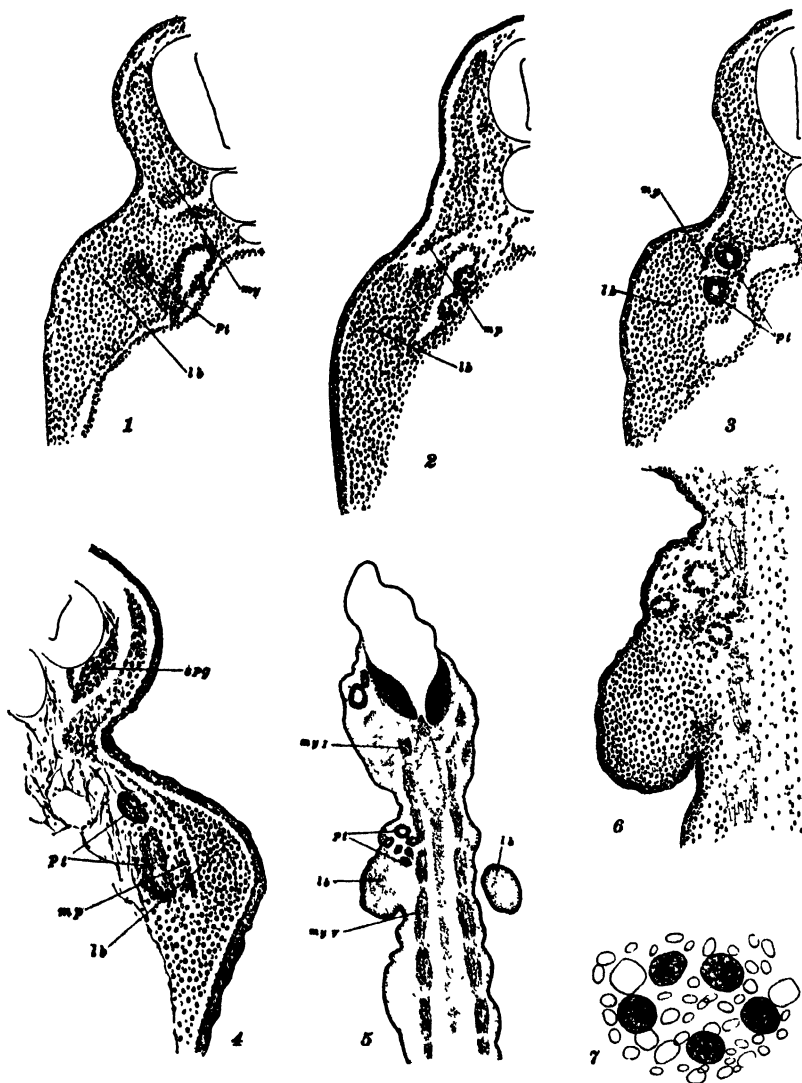


PLATE I

PLATE II

FIG. 8.—Sagittal view through the limb region of a 14 mm. embryo. $\times 19$

FIG. 9.—Transverse section through the limb region of a 14 mm. embryo showing the excessive growth of the pronephric tubules and the separation of the ventral myotome process from its myotome. $\times 47$.

FIG. 10.—Transverse section through the limb bud of a 15 mm. embryo showing the beginning of separation of the mesenchyme cells into the peripheral and axial masses. $\times 47$

FIG. 11.—Transverse section through the limb region of a 16 mm. embryo showing the formation of the dorsal and ventral premuscle masses and the primordial skeletal center. $\times 47$

FIG. 12.—Frontal section through the limb region of a 16 mm. embryo showing the beginning of chondrification in the primordial skeletal center. $\times 47$

FIG. 13.—Frontal section through the pectoral limb of a 17 mm. embryo showing the rudiments of the pectoral girdle and the humerus. $\times 47$

FIG. 14.—Frontal section through the free limb of a 17 mm. embryo showing the primordia of the distal limb skeleton. $\times 47$

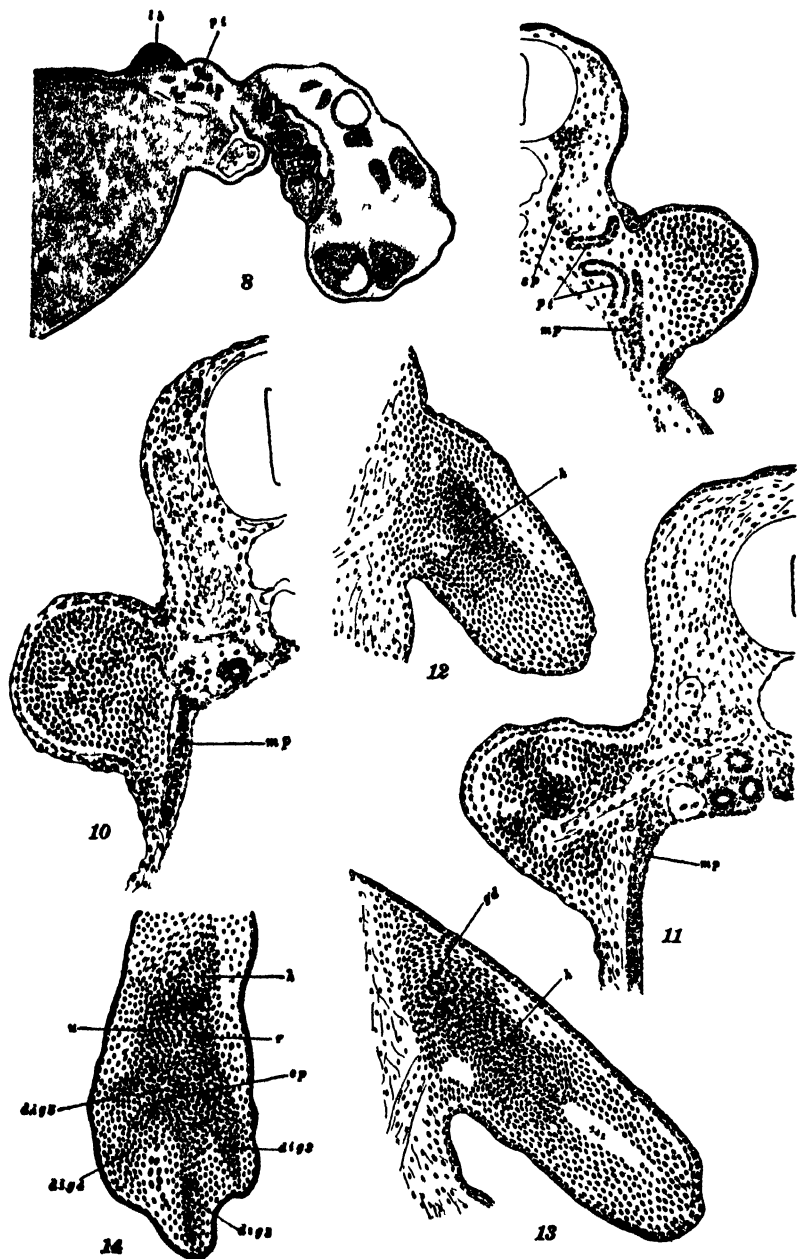


PLATE II

PLATE III

FIGS. 15-17.—Frontal sections through the left pectoral limb of an 18 mm. embryo showing the early formation of the limb skeleton and musculature. $\times 47$

FIG. 18.—Sagittal section showing the centers of chondrification in the pectoral girdle of a 17 mm. embryo. $\times 35$

FIG. 19.—A group of primitive myoblasts in the limb region of a 17 mm. embryo. $\times 350$

FIG. 20.—Myofibrillae without cross striations in the limb bud of a 17 mm. embryo. $\times 240$

FIG. 21.—Cross striated myofibrillae in the limb bud of a 10 mm. embryo. $\times 240$

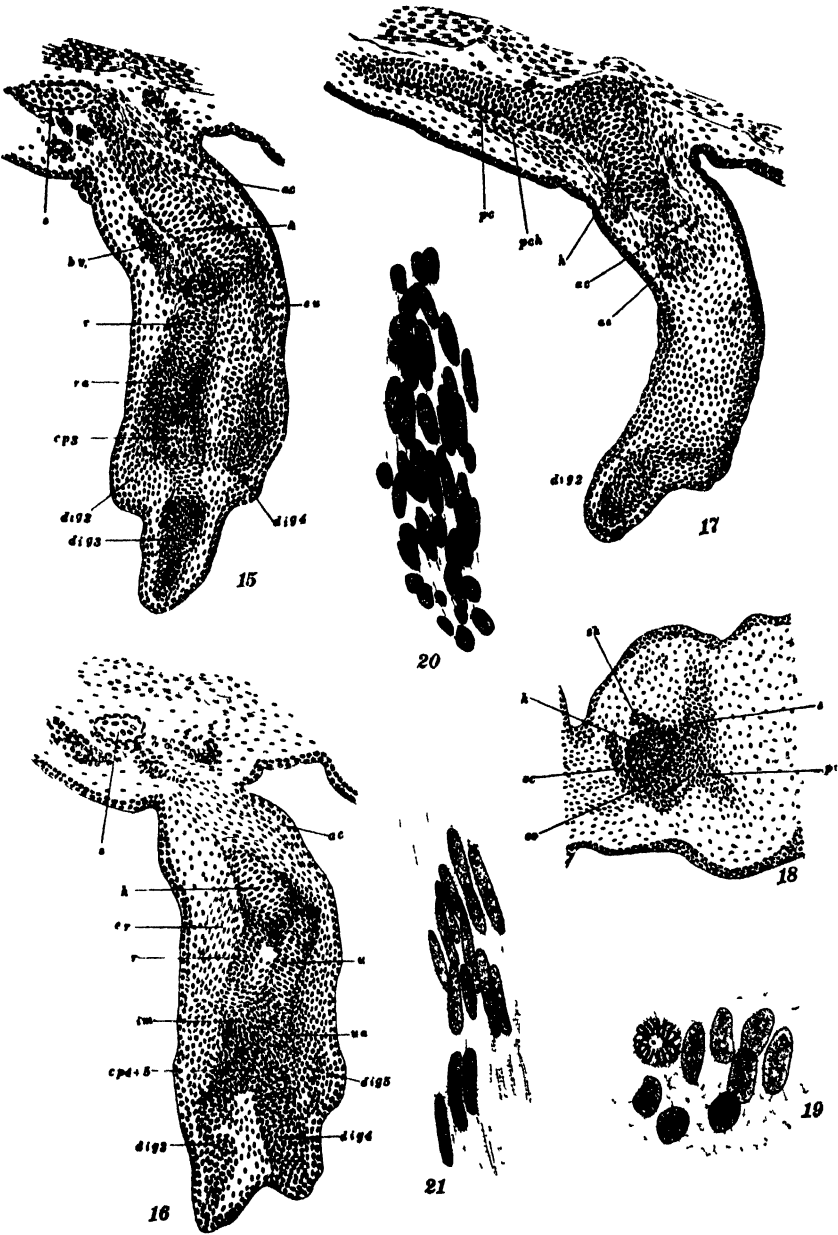


PLATE III

PLATE IV

FIG. 22.—Frontal section through the limb of a 19 mm. embryo. $\times 32$

FIG. 23.—Sagittal section through the pectoral girdle of a 23 mm. embryo. $\times 20$

FIG. 24.—Transverse section through the scapular region of a 19 mm. embryo. $\times 20$.

FIG. 25.—Transverse section through the glenoid cavity of a 19 mm. embryo. $\times 20$

FIG. 26.—Frontal section of the radius of a 23 mm. embryo showing the formation of three zones of chondroblasts. $\times 75$

FIG. 27.—Transverse section of a 9 mm. embryo showing the neural crest cells. $\times 54$

FIG. 28.—Transverse section of a 13 mm. embryo showing the downward growth of a spinal nerve in the limb region. $\times 54$

FIG. 29.—Transverse section of a 19 mm. embryo showing the downward growth of a spinal nerve in the limb region. $\times 54$

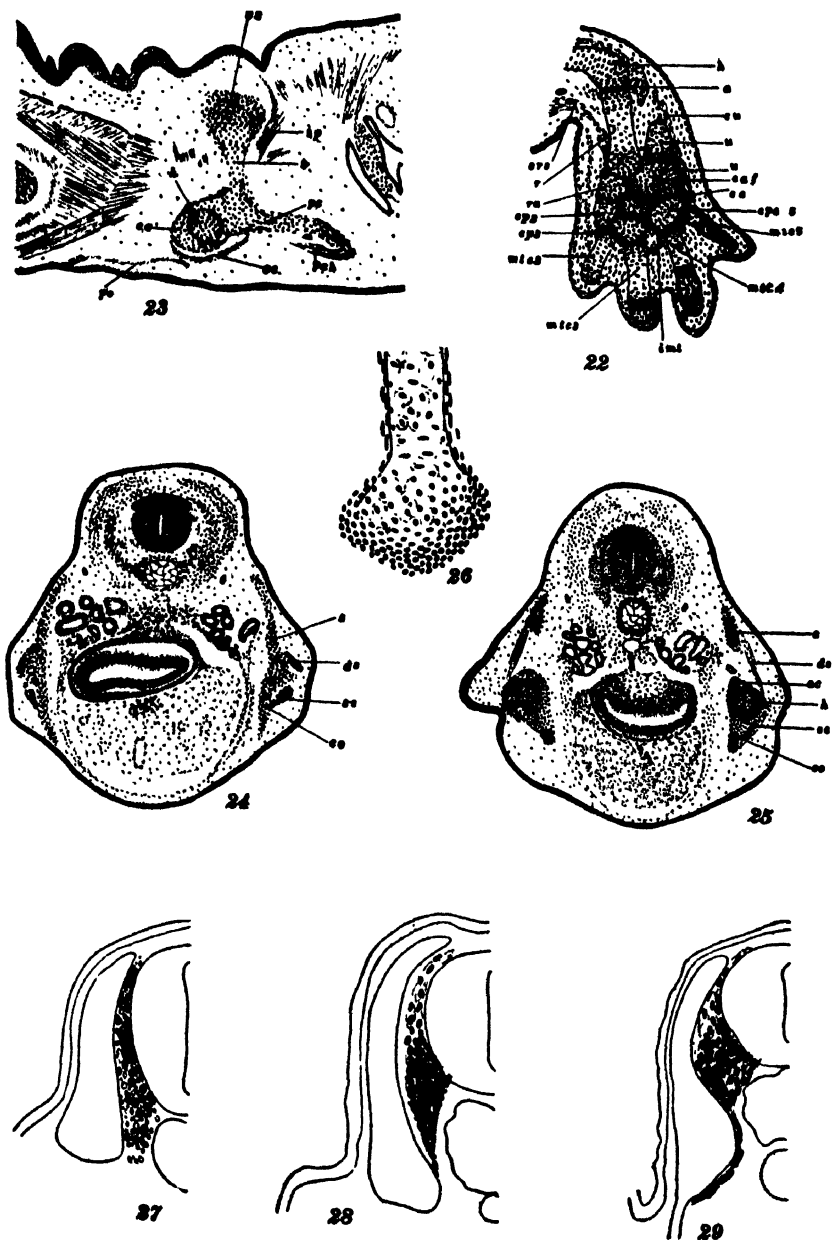


PLATE V

FIG. 30.—Frontal section of a 34 mm. embryo showing three spinal nerves in the limb region. $\times 20$

FIG. 31.—Transverse section through the scapula and supra-scapula of a 32 mm. embryo. $\times 20$

FIG. 32.—Transverse section through the glenoid cavity and the coracoid of a 32 mm. embryo. $\times 20$

FIG. 33.—Frontal section through the procoracoid of a 32 mm. embryo. $\times 20$

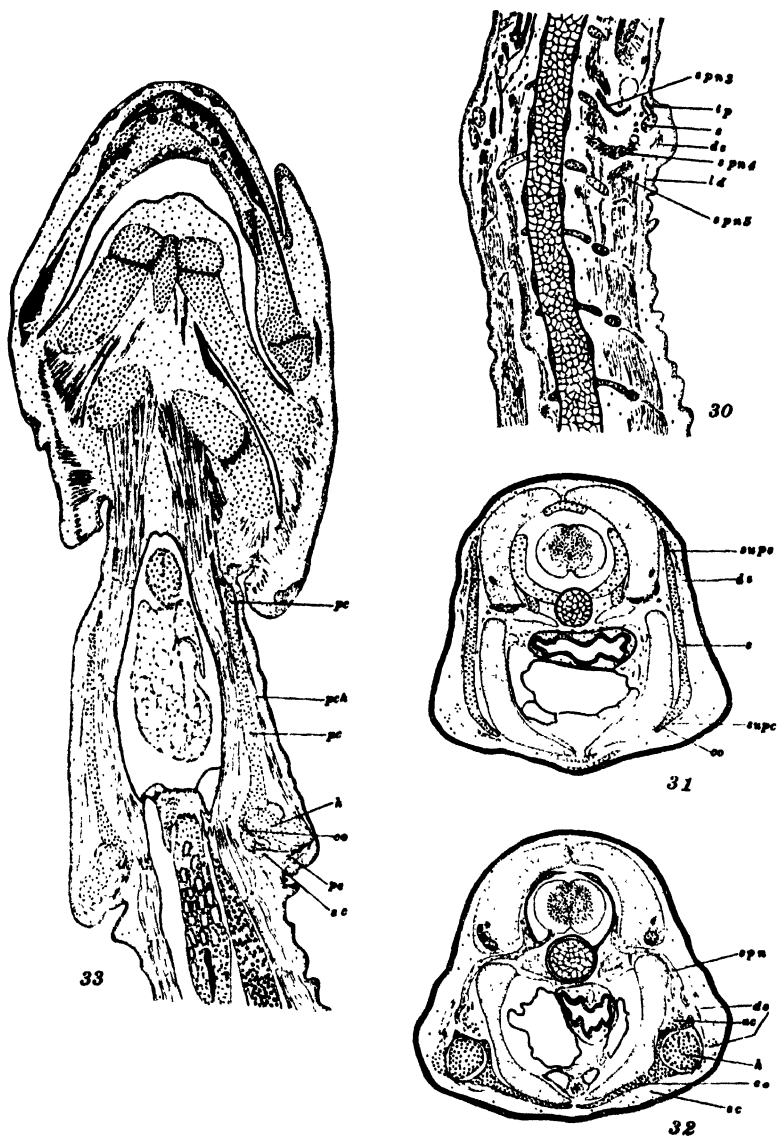


PLATE V

PLATE VI

FIG. 34.—Transverse section of a 30 mm. embryo showing the overlapping of the coracoids. $\times 20$.

FIG. 35.—Transverse section of a 34 mm. embryo showing the humerus and the anconeus muscles. $\times 20$

FIG. 36.—Sagittal sections through the girdle region of a 34 mm. embryo. $\times 14$

FIG. 37.—Sagittal section through the girdle region of a 34 mm. embryo showing the muscles in the girdle region. $\times 14$

FIG. 38.—Frontal section through the supra-scapula of a 23 mm. embryo. $\times 30$

FIG. 39.—Frontal section through the coracoid of a 23 mm. embryo. $\times 35$

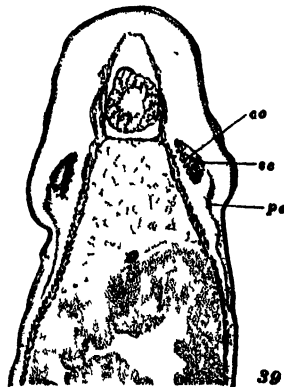
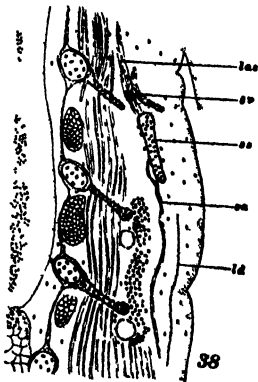
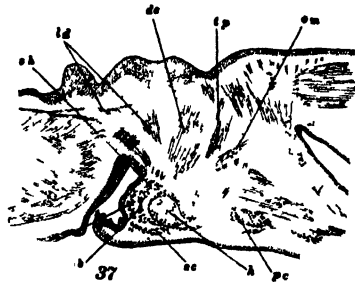
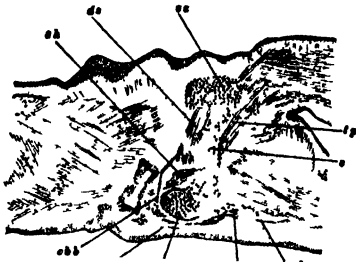
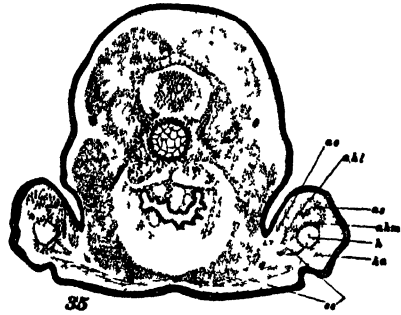
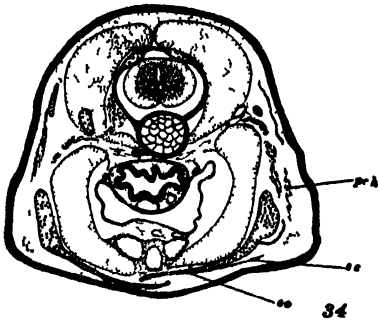


PLATE VI

PLATE VII

FIG. 40.—Frontal section through the flexor surface of the forearm and hand of a 30 mm. embryo. $\times 27$

FIG. 41.—Frontal section through the extensor surface of the forearm and hand of a 21 mm. embryo. $\times 32$

FIG. 42.—Frontal section through the forearm and hand of a 21 mm. embryo. $\times 32$

FIG. 43.—Limb bud of a 13 mm. embryo, length, 0.5 mm.

FIG. 44.—Limb bud of a 15 mm. embryo, length, 0.8 mm.

FIG. 45.—Limb bud of a 16 mm. embryo, length, 0.95 mm.

FIG. 46.—Limb bud of a 17 mm. embryo, length, 1.2 mm.

FIG. 47.—Limb bud of an 18 mm. embryo, length, 1.6 mm.

FIG. 48.—Limb bud of a 19 mm. embryo, length, 1.9 mm.

FIG. 49.—Limb bud of a 20 mm. embryo, length, 2.6 mm.

FIG. 50.—Limb bud of a 24 mm. embryo, length, 3.1 mm.

FIG. 51.—Limb bud of a 33 mm. embryo, length, 3.5 mm.

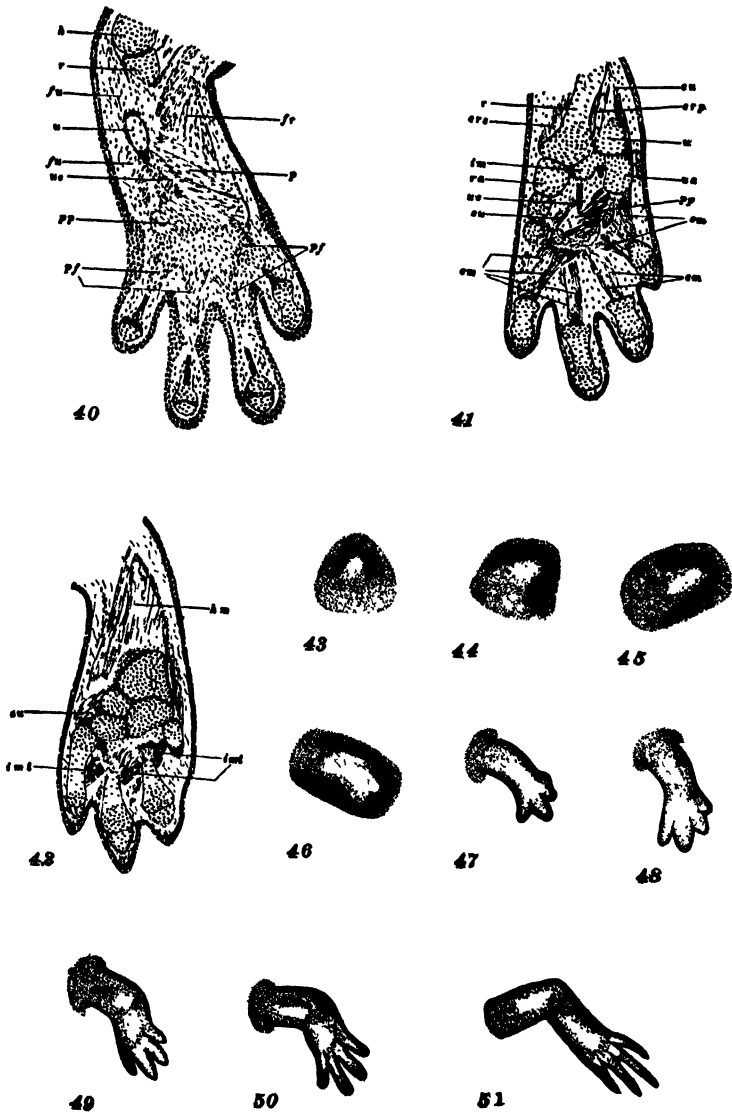


PLATE VII

PLATE VIII

FIG. 52.—Lateral view of a reconstruction of the right pectoral limb of a 17.5 mm. embryo.

FIG. 53.—Median view of the above reconstruction.

FIG. 54.—Lateral view of a reconstruction of the right pectoral limb of a 19 mm. embryo.

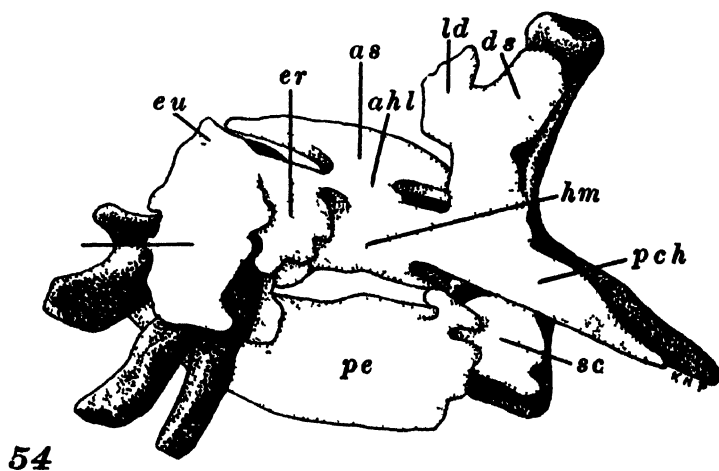
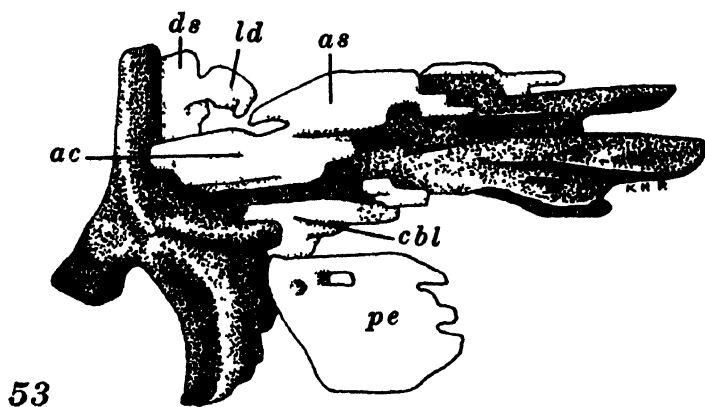
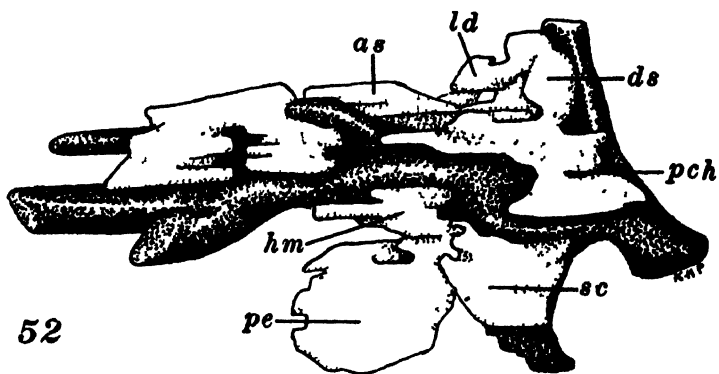


PLATE IX

FIG. 55.—Median view of a reconstruction of the right pectoral limb of a 19 mm. embryo.

FIG. 56.—Median view of a reconstruction of the right pectoral limb of a 21 mm. embryo.

FIG. 57.—Median view of a reconstruction of the right pectoral limb of a 23 mm. embryo.

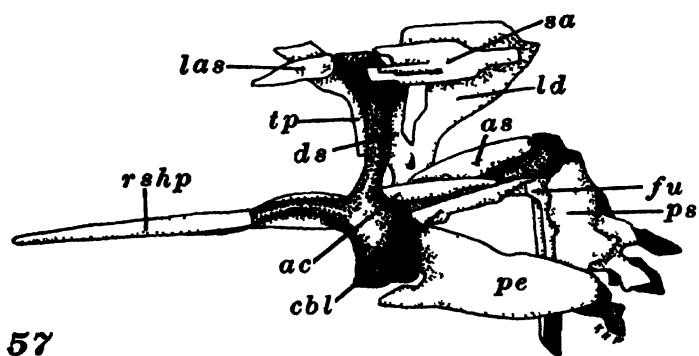
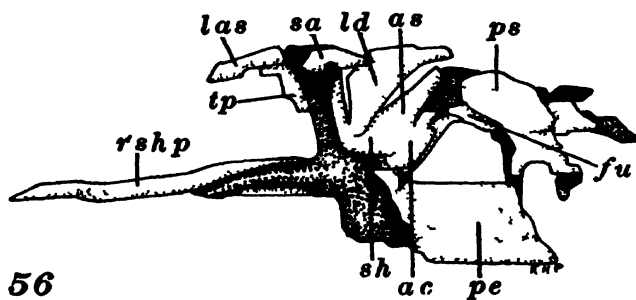
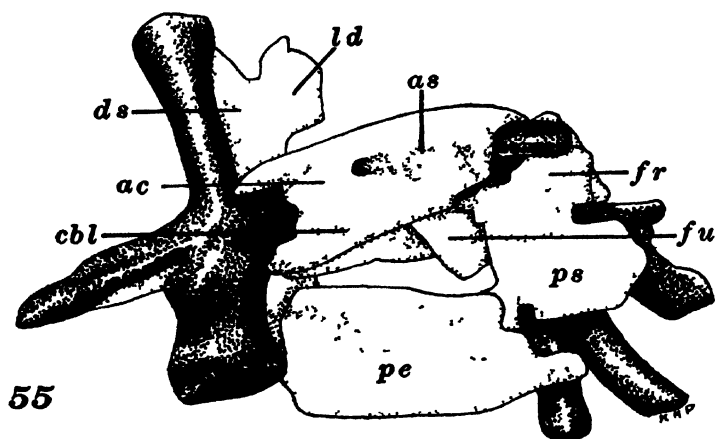


PLATE IX

PLATE X

FIG. 58.—Median view of a reconstruction of the right pectoral limb of a 27 mm. embryo.

FIG. 59.—Lateral view of a reconstruction of the right pectoral limb of a 34 mm. embryo.

FIG. 60.—Reconstruction of the left free limb of a 21 mm. embryo.

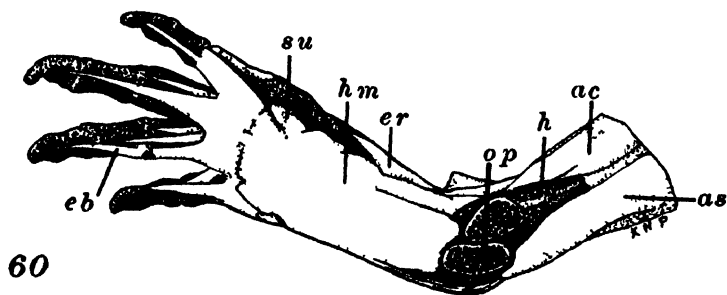
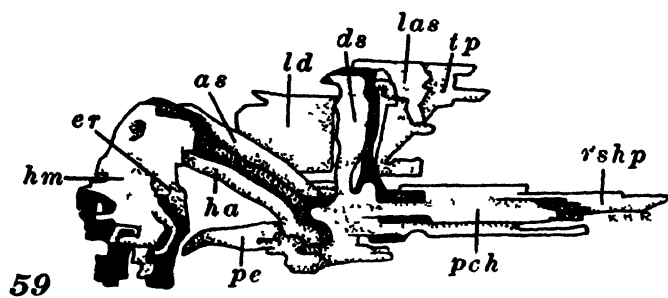
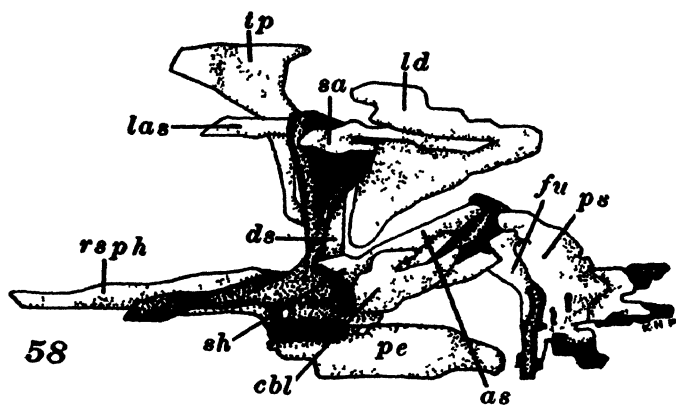


PLATE X

PLATE XI

Figs. 61-63.—Reconstruction of the brachial plexus in a 34 mm. larva.

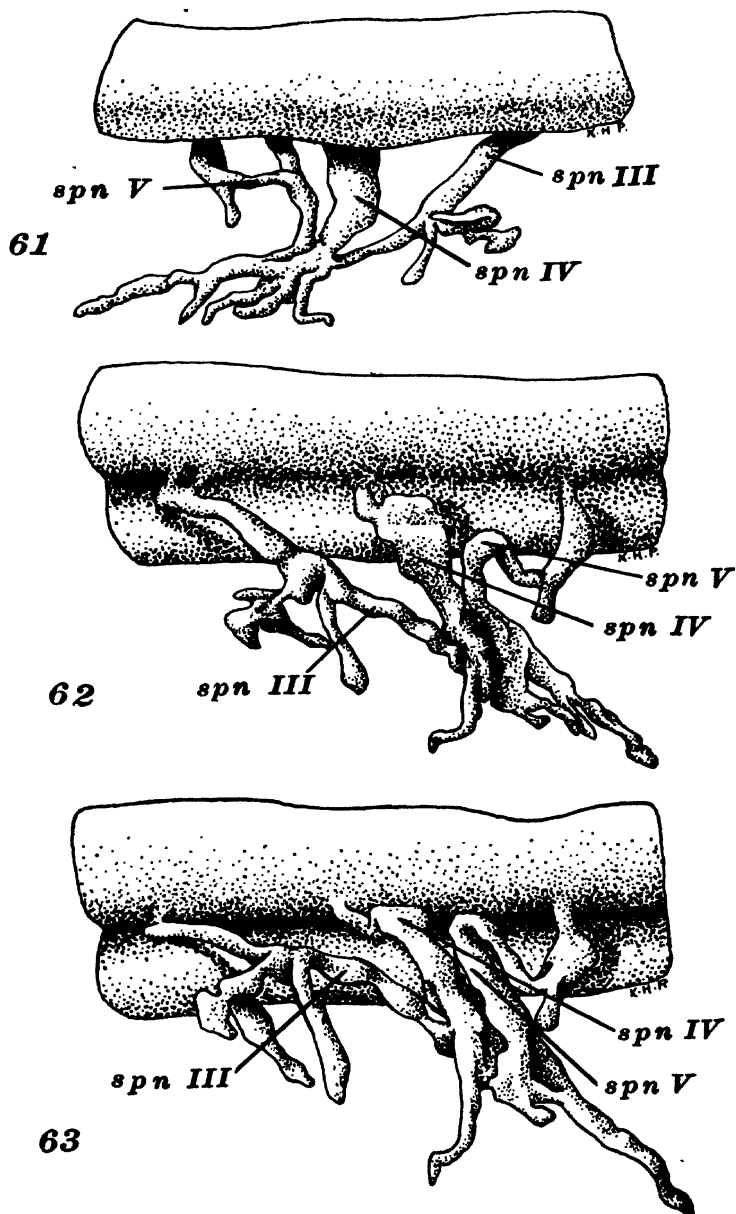


PLATE XI

ILLINOIS BIOLOGICAL MONOGRAPHS

Vol. XIV

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STUDIES ON NORTH AMERICAN CERCARIAE

WITH EIGHT PLATES

By
EDWIN LYNN MILLER

CONTRIBUTION FROM THE ZOOLOGICAL LABORATORY OF THE
UNIVERSITY OF ILLINOIS
No. 475

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To Professor Henry B. Ward, under whose direction the investigations have been carried out, the writer wishes to acknowledge indebtedness for the use of his valuable private library and for his suggestions and helpful criticisms. Also a number of friends and fellow students have aided materially in the identification of mollusks and the collection of material during the course of the following studies.

I. INTRODUCTION*

From the time of Joseph Leidy to comparatively recent years American larval trematodes and trematode life histories have received increasing attention. Before Cort published his pioneer work on larval trematodes of Illinois, very little was known about these larvae in North America, and even in Europe only a few life cycles were known. Up to 1914 only twelve cercariae had been reported from North America, but in the same year Cort added fourteen new species to this list.

Faust described fifteen additional species in 1917 and two years later compiled a list of eighty-one described forms from the United States. Such studies became more prevalent soon after this time, so that McCoy in 1928 estimated the number of described cercariae in this country as about one hundred, but stated that the complete life histories of only four trematodes in the United States were known. Up to 1929 ten complete life histories had been described.

While these estimates may in some cases be incomplete, nevertheless they serve to show one of the trends of modern investigation in the field of Helminthology. Now the number of described cercariae in this country has reached such proportions that it is not practical even to refer to all previous descriptions in this paper. Descriptions from the state of Illinois alone, including those in the present paper, cover thirty-eight species.

In some attempts to establish life histories of these forms, either experimentally or morphologically, many errors have occurred, largely because of unsubstantiated generalizations or insufficient experimentation. Due to a diversity of structure within each of the several larval groups, I find many difficulties are encountered in determining a relationship between the morphology and the developmental cycle. In short, I believe Dollfus (1914) was right when he stated that cercariae very similar in structure dwell in different hosts and have very different kinds of development, and that cercariae very different morphologically live in identical environments and have similar courses of development.

In his classification of cercariae in 1909, Lühe recognized five major groups, Lophocercariae and Gasterostome, Monostome, Amphistome, and Distome Cercariae. He further subdivided the Distome Cercariae into the Cystocercous, Rhopalocercous, Leptocercous, Trichocercous, Furcocercous, Microcercous, and Gorgonocephalous Cercariae, as well as tailless cercariae.

*It is not convenient to consider here several publications which have appeared since this manuscript was completed in 1934.

Lebour (1912) used the development in sporocysts or rediae of these forms as a basic factor in her scheme of classification. Viewing this plan now in the light of numerous additions to taxonomic lists of cercariae from various parts of the world, I realize that such a scheme can denote only very general relationships since individuals of the subgroups within each of these two large groups differ greatly in most other respects.

No one can doubt that an accurate classification of cercariae based purely on relationship would be of great value in indicating possible clues to the life histories of closely related larvae. However, the still inadequate knowledge concerning the value of many larval characters in revealing adult relationships and the inadequate descriptions of many cercariae make such a classification still impossible for all of the described larvae.

Certain larval structures, such as the number and position of the penetration glands, the shape of the bladder, the branching of the excretory system, the shape and size of the stylet, and the character of the germinal mass, should be regarded as less important for definite specific diagnoses and more important for showing subgroup relationships. Earlier workers were sometimes inclined to regard these characters as specific.

Perhaps the statement of Faust (1917) shows adequately the viewpoint taken by some more recent workers, when he says: "The writer has attacked this part of the problem with the idea in mind that not only the fundamentals of the adult trematode are found in the mature cercaria, but that even the main descriptive features of the adult trematode are already present, so that the worker can recognize the adult in the larva." After various studies on many cercariae I am inclined to depart somewhat from the above viewpoint, for while many of the larval characters resemble closely those of the adult, yet I find several incompletely developed structures in the cercaria which bear little resemblance to the same structures in the adult. For instance, the poorly defined and shapeless germinal mass of the emerged furcocercous cercaria bears little resemblance to the reproductive organs of the adult trematode. The character of the excretory system may change due to the division of the flame cells and the growth of tubules and capillaries, and even a ventral sucker may appear during the metacercarial stage (McCoy, 1929). I believe the above facts must be considered carefully if we are to progress toward the use of dependable characters of the cercaria for specific diagnosis.

Similarity between excretory systems has proved to be of great value in determining relationships but I find this similarity too great to have the systematic significance given it by some workers. Several instances of different species of cercariae, as well as different species of

adults, are on record in which the excretory systems are identical as to the arrangements of the tubules and terminal cells.

The discovery that many cercariae do not have the host-specificity originally supposed has brought to light almost identical descriptions of forms from different molluscan hosts.

The following studies may help in clarifying some of the earlier errors in taxonomy and descriptions of North American cercariae, and will add to the information concerning the life histories of several forms. Descriptions of four new cercariae are included in the morphological section of this paper, and I have added to the knowledge of nineteen additional species.

It is hoped that the survey of the Illinois forms and the summary of the localities and hosts from which each form has been reported may be of use to future investigators.

II. METHODS OF INVESTIGATION

Former workers who have placed emphasis on the study of living forms have recognized the advantages in such methods. Earlier descriptions of cercariae based on preserved and mounted material are responsible for much of the confusion existing in the literature at present. Such descriptions have made it impossible to determine the identity of many such forms with certainty and have made their inclusion in identification keys and systems of classification highly problematical.

As pointed out in my earlier paper (1930), technique methods do not insure a similarity in the proportions of body structures before and after killing and fixing. Obviously the study of such material cannot give a true and complete concept of these forms.

For these reasons the great majority of my investigations have been based on living specimens, and detailed studies of all forms included here have been made on the living animals with the aid of an oil immersion. Pressure of the cover slip is advantageous since it makes structures more visible. It also slows down the activity of the worm so that more accurate measurements can be completed on the living form. Most of the following observations were made on living cercariae under uniform pressure which was just strong enough to prevent rapid movement.

Measurements without explanations of the stages of contraction or expansion of the body mean very little. Therefore I have tried particularly to obtain measurements at various stages of body contraction and extension. Only in this way can such measurements be of use to future investigators. Abundant material for the study of local forms has made

this possible. All measurements mentioned here, unless otherwise specified, were made from living material, and represent the averages for at least five different individuals.

Recent workers have shown the error in basing descriptions on only partially developed cercariae which have been secured by dissection of the molluscan host. The following morphological studies have been made from cercariae after emergence from the mollusks. However, Stunkard (1930) has found that cercariae may leave the sporocyst or redia while still immature, and complete their development in the lymph spaces of the mollusk. My own observations have corroborated this, and, in addition, there is evidence that at least some of the stylet cercariae, under conditions of high temperature in small pools during the hot summer months, emerge from the snail prematurely. In such cases there are several points of difference between their structure and that of forms which emerge during the colder fall and spring days. It has been suggested in recent descriptions (Beaver, 1929; Horsfall, 1930) that some earlier descriptions have been based on such immature forms.

It would seem that abnormal conditions as yet little understood are at work, when observations show that at least with some species, those leaving the snail soon after it has been placed in a laboratory aquarium encyst normally, but those appearing later due to this artificial rise in temperature die without encysting. Perhaps their development has been speeded to such an extent that they break from the sporocyst and emerge from the snail prematurely, death consequently resulting. This may also explain why many hosts remain uninfected during the experimental life history studies of cercariae.

As indicated in the tables of collection records and in the collection dates in the description of each species included in this paper, my work extended over the period from September, 1931, to July, 1933. In the laboratory the snails were isolated in small vials or bottles and examined repeatedly for about forty-eight hours. Those not giving off cercariae were later examined for infections so that the rediae or sporocysts and developing cercariae might be studied. Infected individuals were segregated in small containers so that the movements of the emerging cercariae could be studied before microscopical studies were made. Later, cysts were recovered for study from the inner walls of these containers and from the snails themselves since they often serve as the host of the encysted forms as well, particularly for the echinostome cercariae.

Incidence of emergence could be studied too, and has been mentioned in the descriptions of some species. Parasitized snails frequently die in the laboratory, although, in some cases, it has been possible to keep severely parasitized hosts for long periods.

Cercariae were fixed with warm Gilson's fluid, saturated corrosive sublimate fixing fluid, and corrosive acetic. Sections of the liver, including sporocysts or rediae, were also fixed and in all cases preserved in 75% alcohol. Entire mounts of the cercariae, sporocysts, and rediae were made for additional study and later reference, after staining with Delafield's hematoxylin, Ehrlich's acid hematoxylin, Mayer's paracarmine, alum cochineal, and Mallory's phospho-tungstic hematoxylin.

In addition to the methods mentioned above, intra-vitam staining was used in these studies. The use of neutral red for staining certain glands and organs in the living animal proved to be very valuable, particularly for distinguishing the number of penetration glands and the slender, undeveloped ceca of the digestive system, both important points in identification. Weak solutions of methylene blue, introduced under the coverslip, aided in locating spines of the tail and body, and the collar spines of echinostome cercariae.

III. DISCUSSION OF INFECTION RECORDS

An examination of the data on molluscan infection with the cercariae discussed in this survey brings out a number of interesting features of the work. As previously mentioned, the reporting of many molluscan hosts for the same species of cercaria in the same locality has been questioned. However, Cort (1918) gave several striking examples of the lack of specificity in the choice of intermediate hosts, notably the human schistosome cercariae, *C. douthitti* Cort 1914 and *C. douglasi* Cort 1917. He submitted data to support this contention and said: "The data given above seems to clearly indicate that the forked-tailed cercariae readily adapt themselves to new molluscan intermediate hosts."

Many species have now been reported from a wide variety of hosts. From my own records I can say with certainty that *C. acanthocoela* has been found in *Physa gyrina hildrethiana* and *Helisoma trivolvis*; that *C. mesotylpha* occurs in *Physa gyrina hildrethiana*, *P. halei*, and *P. gyrina*; that *C. pteractinota* infests both *P. gyrina* and *H. trivolvis*; and that *C. trivolvis* Cort 1914 was found in both *H. trivolvis* and *P. gyrina*. In fact the very existence of many forms depends upon their cosmopolitan adjustment to various hosts in different localities. This has been proved by distributional and experimental studies.

Miller and Northup (1926) concluded that there is a semi-annual rise and fall in the larval trematode infestation of *Nassa obsoleta*. They believed this was due to the migration of the definitive hosts and the degree of their infestation, as well as the life span of *Nassa* and the

effect of parasitism upon it. While my collections were not regular enough to base such conclusions on them, there is some evidence in Table I to show the effect of the long severe summer drouth of 1931 on the larval trematode fauna of gastropods. The instability of this fauna in a particular body of water is also apparent. No doubt many of the holostome cercariae, as well as others, reach local bodies of water through the infected excrement of migrant or summer-resident birds. A certain species of cercaria may be found only once in a given locality, even though collections are continued over a period of several years. But even though many of the species studied are isolated in particular bodies of water, several forms, such as *C. pteractinota*, *C. trivolvis*, and *C. meso-typhla* appear to be distributed generally over the area represented by the collections (see Table II).

The data offered by former workers to show the percentage of infection which they found for larval trematodes were a compilation showing the total number of infected snails, that is, both those with undeveloped cercariae and those with emerging cercariae. My data represent an attempt to determine the periods when emergence is normally at its height. Therefore, infections showing only completely developed cercariae are included in the following tables and are represented in the computations of Table IV.

Cort (1922) gave some idea of the enormous number of cercariae that may emerge from a single snail host. He also showed the definite relation between the number of sporocysts present in the snail and its output of cercariae. Temperature has been shown by various workers to be an important regulator of the emergence of cercariae from their hosts. Studies of infected snails after they have been kept in the laboratory for a long period without feeding, indicate that the number of escaping cercariae is greatly reduced by starving the host. In these cases the sporocysts have always contained relatively few cercariae, and in many cases I have noticed dead cercariae and disintegrating germ balls within the cavity of the sporocyst or redia.

An examination of Table IV shows that *Cercaria meniscadena* is the commonest form found in the Urbana area, since over 10% of the *Pleurocera acuta* collected were infected with emerging cercariae. In all, emerging cercariae from about one hundred snails were studied. These infected snails represent about 2.98% of the total number collected from all localities. A few snails were collected at Baton Rouge, Louisiana, Sinking Creek and St. Charles, Missouri, and Leesburg, Florida. These localities have been clearly indicated in Tables I and II. All other collections were made in Illinois, chiefly in the vicinity of the University of Illinois at Urbana.

TABLE I.—COLLECTION RECORDS INCLUDED IN THIS STUDY
(Only completely developed cercariae are listed.)

Date	Place at Which Collected	Collections of Mollusks			Species of Cercariae described in this paper
		Scientific Name	Number Collected	Number Infected	
9/21/31.....	Oxbow, Urbana	<i>Physa gyrina hildrethiana</i>	32	0	<i>C. mesotrypha</i> E. L. Miller 1935
		<i>Helisoma trivolvis</i>	33	0	
9/22/31.....	Oxbow, Urbana	<i>P. gyrina hildrethiana</i>	36	0	
		<i>H. trivolvis</i>	10	0	
9/24/31.....	Oxbow, Muncie	<i>P. gyrina hildrethiana</i>	107	1	
9/26/31.....	Pond, Mahomet	<i>H. trivolvis</i>	18	0	<i>C. meniscadena</i> E. L. Miller 1935
		<i>P. gyrina hildrethiana</i>	6	0	
9/29/31.....	Salt Fork River, Homer	<i>Goniobasis livescens</i>	27	0	
9/30/31.....	Caldwell's Lake, Seymour	<i>P. gyrina hildrethiana</i>	5	0	
10/2/31.....	Sangamon River, Mahomet	<i>Pleurocera acuta</i>	20	0	
10/5/31.....	Sangamon River, Mahomet	<i>P. acuta</i>	177	23	<i>C. pteractinota</i> E. L. Miller 1935
10/8/31.....	Twin Lakes, Paris	<i>Physa gyrina</i>	26	1	
		<i>H. trivolvis</i>	5	0	

TABLE I.—COLLECTION RECORDS INCLUDED IN THIS STUDY (*Continued*)
(Only completely developed cercariae are listed.)

Date	Place at Which Collected	Collections of Mollusks			Species of Cercariae described in this paper
		Scientific Name	Number Collected	Number Infected	
10/8/31.....	Cole's Pond, Charleston	<i>Succinea ovalis</i>	2	1	<i>L. problematicum</i> Magath 1920
10/21/31.....	Oxbow, Urbana	<i>P. gyrina hildrethiana</i>	28	0	<i>C. hamata</i> Miller 1923
		<i>H. trivolvis</i>	156	2	
10/22/31.....	Twin Lakes, Paris Cole's Pond, Charleston	<i>P. gyrina</i>	76	0	<i>C. pteractinota</i> E. L. Miller 1935
		<i>P. gyrina</i>	12	0	
		<i>H. trivolvis</i>	12	0	
		<i>H. trivolvis</i>	12	1	
10/23/31.....	Drainage Ditch, Urbana				<i>C. acanthocoela</i> E. L. Miller 1935
10/26/31.....	Camp Creek, Seymour	<i>P. gyrina hildrethiana</i>	74	1	<i>C. mesotophla</i> E. L. Miller 1935
				8	<i>C. urbanensis</i> Cort 1914
			37	1	<i>C. pteractinota</i> E. L. Miller 1935
		<i>H. trivolvis</i>		1	<i>C. trivolvis</i> Cort 1914
				1	<i>C. packycystata</i> E. L. Miller 1935

TABLE I.—COLLECTION RECORDS INCLUDED IN THIS STUDY (*Continued*)
(Only completely developed cercariae are listed.)

Date	Place at Which Collected	Collections of Mollusks			Species of Cercariae described in this paper
		Scientific Name	Number Collected	Number Infected	
10/27/31.....	Camp Creek, Seymour	<i>P. gyrina hildrethiana</i>	221	4	<i>C. urbanensis</i> Cort 1914
10/28/31.....	Leesburg, Florida	<i>Ampullaria depressa</i>	3	1	<i>C. cystonchoides</i> E. L. Miller 1935
		<i>Viciparus georgiana</i>	175	3	<i>C. cyclica</i> nov. sp.
11/1/31.....	Sangamon River, Mahomet Camp Creek, Seymour	<i>P. acuta</i> <i>H. trivolvis</i>	7 30	0 1	<i>C. acanthocoela</i> E. L. Miller 1935
11/4/31.....	Sangamon River, Mahomet	<i>Actinonaias carinata</i>	2	1	<i>C. mitocerca</i> E. L. Miller 1935
11/10/31.....	Lake Decatur, Decatur	<i>P. gyrina hildrethiana</i>	512	2	<i>C. hamata</i> Miller 1923
11/18/31.....	Kaskaskia River, Sadorus	<i>P. gyrina hildrethiana</i>	39	0	
11/24/31.....	Oxbow, St. Joseph	<i>Musculium transversum</i>	28	2	<i>C. sphaerocerca</i> E. L. Miller 1935
12/8/31.....	Oxbow, St. Joseph	<i>P. gyrina hildrethiana</i>	12	0	

TABLE I.—COLLECTION RECORDS INCLUDED IN THIS STUDY (Continued)
(Only completely developed cercariae are listed.)

Date	Place at Which Collected	Collections of Mollusks			Species of Cercariae described in this paper
		Scientific Name	Number Collected	Number Infected	
12/28/31.....	Sangamon River, Mahomet	<i>Campeloma</i> sp.	18	0	<i>C. acanthocoela</i> E. L. Miller 1935 <i>C. multicellulata</i> Miller 1923 <i>C. steganocoela</i> E. L. Miller 1935
4/4/32.....	Camp Creek, Seymour	<i>P. gyrina hildrethiana</i>	65	1	
				1	
				1	
4/8/32.....	Cole's Stream, St. Charles, Missouri	<i>Physa halei</i>	126	0	<i>C. mesotrypha</i> E. L. Miller 1935
	Alhambra	<i>P. halei</i>	140	1	
4/10/32.....	Camp Creek, Seymour	<i>P. gyrina hildrethiana</i>	8	0	
4/15/32.....	Sinking Creek, Mo., Shannon County	<i>Goniobasis</i> sp.	61	0	
4/19/32.....	Oxbow, Urbana	<i>H. trivolvis</i>	12	0	<i>C. acanthocoela</i> E. L. Miller 1935
4/21/32.....	Pollywogs, St. Joseph	<i>P. gyrina</i>	52	0	
		<i>H. trivolvis</i>	18	1	

TABLE I.—COLLECTION RECORDS INCLUDED IN THIS STUDY (Continued)
(Only completely developed cercariae are listed.)

Date	Place at Which Collected	Collections of Mollusks			Species of Cercariae described in this paper
		Scientific Name	Number Collected	Number Infected	
4/26/32.....	Baton Rouge, Louisiana	<i>Helisoma lantum</i>	28	1	<i>C. tridena</i> nov. sp. <i>C. bessiae</i> Cort and Brooks 1928 <i>C. fercalineata</i> nov. sp.
5/2/32.....	Stream, Muncie	<i>P. gyrina</i>	138	0	
5/5/32.....	East Lake Fork, Sadorus	<i>H. trivolvis</i>	58	0	
		<i>P. gyrina hildrethiana</i>	352	1	<i>C. mesotrypha</i> E. L. Miller 1935 <i>C. steganocoela</i> E. L. Miller 1935
5/8/32.....	Sewage Ditch, Urbana	<i>P. gyrina hildrethiana</i>	7	1	<i>C. steganocoela</i> E. L. Miller 1935
		<i>Fossaria obrussa</i>	17	0	
	Pond No. 4, Urbana	<i>H. trivolvis</i>	12	0	
		<i>P. gyrina</i>	11	0	
		<i>Lymnaea</i> sp.	11	0	

TABLE I.—COLLECTION RECORDS INCLUDED IN THIS STUDY (*Continued*)
(Only completely developed cercariae are listed.)

Date	Place at Which Collected	Collections of Mollusks			Species of Cercariae described in this paper
		Scientific Name	Number Collected	Number Infected	
5/12/32.....	Salt Fork River, Homer	<i>G. livescens</i>	265	10	<i>C. cystorhysa</i> E. L. Miller 1935
5/29/32.....	Sangamon River, Mahomet	<i>P. gyrina</i>	2	1	<i>C. gigas</i> Faust 1918
6/8/32.....	Oxbow, St. Joseph	<i>P. gyrina</i>	11	1	<i>C. mesotyphla</i> E. L. Miller 1935
		<i>H. trivolvis</i>	10	1	<i>C. trivolvis</i> Cort 1914
6/12/32.....	Mud Slough, Henry	<i>H. trivolvis</i>	12	1	<i>C. pachycystata</i> E. L. Miller 1935
6/14/32.....	Oxbow, St. Joseph	<i>P. gyrina</i>	3	2	<i>C. mesotyphla</i> E. L. Miller 1935
		<i>H. trivolvis</i>	35	1	<i>C. trivolvis</i> Cort 1914
				2	<i>C. hamata</i> Miller 1923
					<i>C. pteractinota</i> E. L. Miller 1935

TABLE I.—COLLECTION RECORDS INCLUDED IN THIS STUDY (Concluded)
(Only completely developed cercariae are listed.)

Date	Place at Which Collected	Collections of Mollusks			Species of Cercariae described in this paper
		Scientific Name	Number Collected	Number Infected	
6/18/32.....	Baton Rouge, Louisiana	<i>H. lantum</i>	11	1	<i>C. tricystica</i> E. L. Miller 1935
6/19/32.....	Baton Rouge, Louisiana	<i>P. gyrina</i>	14	1	<i>C. pteractinola</i> E. L. Miller 1935
				1	<i>C. louisiana</i> E. L. Miller 1935
6/25/32.....	Oxbow, St. Joseph	<i>H. trivolvis</i>	90	1	<i>C. acanthococlea</i> E. L. Miller 1935
7/7/32.....	Pond, Rantoul	<i>P. gyrina</i>	12	3	<i>C. mesotylpha</i> E. L. Miller 1935
	Oxbow, St. Joseph	<i>H. trivolvis</i>	14	6	<i>C. trivolvis</i> Cort 1914
7/9/32.....	Oxbow, St. Joseph	<i>H. trivolvis</i>	26	2	<i>C. wardi</i> Miller 1923
				1	<i>C. trivolvis</i> Cort 1914
				1	<i>C. hamata</i> Miller 1923
				1	<i>C. acanthococlea</i> E. L. Miller 1935
		<i>P. gyrina</i>	5	1	<i>C. trivolvis</i> Cort 1914

TABLE II.—REGIONS OF SPECIFIC CERCARIAL INFESTATIONS

Locality	Molluscan Hosts	Cercariae
Alhambra	<i>Physa halei</i>	<i>C. mesotyphla</i>
Baton Rouge, Louisiana	<i>Helisoma lantum</i>	<i>C. tricystica</i> <i>C. tridena</i> <i>C. bessiae</i> <i>C. furcalineata</i> <i>C. pteractinota</i> <i>C. louisiana</i>
	<i>Physa gyrina</i>	
Caldwell's Lake, Seymour	<i>Physa gyrina hildrethiana</i>	None
Camp Creek, Seymour	<i>Physa gyrina hildrethiana</i>	<i>C. acanthocoela</i> <i>C. multcellulata</i> <i>C. steganocoela</i> <i>C. mesotyphla</i> <i>C. urbanensis</i> <i>C. pteractinota</i> <i>C. trivolvis</i> <i>C. pachycystata</i> <i>C. acanthocoela</i>
	<i>Helisoma trivolvis</i>	
Cole's Pond, Charleston	<i>Succinea ovalis</i>	<i>L. problematicum</i>
	<i>Physa gyrina</i>	None
	<i>Helisoma trivolvis</i>	None
Cole's Stream, St. Charles, Missouri	<i>Physa crandalli</i>	None
Drainage Ditch, Urbana	<i>Helisoma trivolvis</i>	<i>C. acanthocoela</i>
East Lake Fork, Sadorus	<i>Physa gyrina hildrethiana</i>	<i>C. mesotyphla</i> <i>C. steganocoela</i>
	<i>Helisoma trivolvis</i>	None
Kaskaskia River, Sadorus	<i>Physa gyrina hildrethiana</i>	None
Lake Decatur, Decatur	<i>Physa gyrina hildrethiana</i>	<i>C. hamata</i>
Leesburg, Florida	<i>Ampullaria depressa</i>	<i>C. cystonchnoides</i>
	<i>Viviparus georgiana</i>	<i>C. cyclica</i>
Mud Slough, Henry	<i>Helisoma trivolvis</i>	<i>C. pachycystata</i>
Oxbow, Muncie	<i>Physa gyrina hildrethiana</i>	<i>C. mesotyphla</i>

TABLE II.—REGIONS OF SPECIFIC CERCARIAL INFESTATIONS (*Concluded*)

Locality	Molluscan Hosts	Cercariae
Oxbow, St. Joseph	<i>Musculium transversum</i> <i>Physa gyrina hildrethiana</i> <i>Physa gyrina</i> <i>Helisoma trivolvis</i>	<i>C. sphaerocerca</i> None <i>C. trivolvis</i> <i>C. mesotyphla</i> <i>C. trivolvis</i> <i>C. hamata</i> <i>C. pteractinota</i> <i>C. acanthocoela</i> <i>C. wardi</i>
Oxbow, Urbana.....	<i>Physa gyrina hildrethiana</i> <i>Physa gyrina</i> <i>Helisoma trivolvis</i>	None None <i>C. hamata</i> <i>C. pteractinota</i>
Pollywogs, St. Joseph	<i>Physa gyrina</i> <i>Helisoma trivolvis</i>	None <i>C. acanthocoela</i>
Pond, Mahomet.....	<i>Helisoma trivolvis</i> <i>Physa gyrina hildrethiana</i>	None None
Pond No. 4, Urbana	<i>Helisoma trivolvis</i> <i>Physa gyrina</i> <i>Lymnaea sp.</i>	None None None
Pond, Rantoul	<i>Physa gyrina</i>	<i>C. mesotyphla</i>
Salt Fork River, Homer	<i>Goniobasis livescens</i>	<i>C. cystorhysa</i>
Sangamon River, Mahomet	<i>Pleurocera acuta</i> <i>Actinonaias carinata</i> <i>Cameloma sp.</i> <i>Physa gyrina</i>	<i>C. meniscadena</i> <i>C. mitocerca</i> None <i>C. gigas</i>
Sinking Creek, Missouri, Shannon County.....	<i>Goniobasis sp.</i>	None
Sewage Ditch, Urbana.....	<i>Fossaria obrussa</i> <i>Physa gyrina hildrethiana</i>	None <i>C. steganocoela</i>
Stream, Muncie.....	<i>Physa gyrina</i>	None
Twin Lakes, Paris.....	<i>Physa gyrina</i> <i>Helisoma trivolvis</i>	<i>C. pteractinota</i> None

TABLE III.—PERCENTAGE OF INFECTION OF EACH MOLLUSK HOST WITH EACH CERCARIA STUDIED

Cercaria	Molluscan Host	Number Collected	Number Infected	Percentage of Infection
<i>C. acanthocoela</i>	<i>Physa gyrina hildrethiana</i>	1492	1	0.07
<i>C. acanthocoela</i>	<i>Helisoma trivolvis</i>	600	5	0.83
<i>C. bessiae</i>	<i>Helisoma lantum</i>	39	2	5.13
<i>C. cyclica</i>	<i>Viviparus georgiana</i>	175	3	1.71
<i>C. cystonchnoides</i>	<i>Ampullaria depressa</i>	3	1	33.3
<i>C. cystorhysa</i>	<i>Goniobasis livescens</i>	291	10	3.44
<i>C. furcalineata</i>	<i>Helisoma lantum</i>	39	1	2.57
<i>C. gigas</i>	<i>Physa gyrina</i>	232	1	0.43
<i>C. hamata</i>	<i>Helisoma trivolvis</i>	600	3	0.50
<i>C. hamata</i>	<i>P. gyrina hildrethiana</i>	1492	2	0.13
<i>C. louisiana</i>	<i>Physa gyrina</i>	232	1	0.43
<i>C. meniscadena</i>	<i>Pleurocera acuta</i>	228	23	10.09
<i>C. mesotyphla</i>	<i>Physa gyrina hildrethiana</i>	1492	3	0.20
<i>C. mesotyphla</i>	<i>Physa halei</i>	266	1	0.38
<i>C. mesotyphla</i>	<i>Physa gyrina</i>	232	6	2.59
<i>C. mitocerca</i>	<i>Actinonaias carinata</i>	2	1	50.00
<i>C. multicellulata</i>	<i>Physa gyrina hildrethiana</i>	1492	1	0.07
<i>I. problematicum</i>	<i>Succinea ovalis</i>	2	1	50.00
<i>C. pteractinota</i>	<i>Physa gyrina</i>	232	2	0.86
<i>C. pteractinota</i>	<i>Helisoma trivolvis</i>	600	3	0.50
<i>C. sphaerocerca</i>	<i>Musculium transversum</i>	28	2	7.14
<i>C. steganocoela</i>	<i>Physa gyrina hildrethiana</i>	1492	3	0.20
<i>C. pachycystata</i>	<i>Helisoma trivolvis</i>	600	2	0.33
<i>C. tricystica</i>	<i>Helisoma lantum</i>	39	1	2.57
<i>C. tridena</i>	<i>Helisoma lantum</i>	39	1	2.57
<i>C. trivolvis</i>	<i>Helisoma trivolvis</i>	600	10	1.67
<i>C. trivolvis</i>	<i>Physa gyrina</i>	232	1	0.43
<i>C. urbanensis</i>	<i>Physa gyrina hildrethiana</i>	1492	12	0.83
<i>C. wardi</i>	<i>Helisoma trivolvis</i>	600	2	0.33

TABLE IV.—PERCENTAGE OF CERCARIAL INFECTION FOR EACH SPECIES OF SNAIL HOST

Snail Host	Number Collected	Number Infected	Percentage
<i>Physa gyrina hildrethiana</i>	1492	22	1.47
<i>Helisoma trivolvis</i>	600	23	3.83
<i>Helisoma lantum</i>	39	5	13.00
<i>Viviparus georgiana</i>	175	3	1.14
<i>Ampullaria depressa</i>	3	1	33.33
<i>Goniobasis livescens</i>	291	10	3.44
<i>Pleurocera acuta</i>	228	23	10.08
<i>Physa gyrina</i>	232	11	4.73
<i>Physa halei</i>	266	1	.38
Total	3326	99	Average 2.98

IV. DISCUSSION AND KEY TO THE
ILLINOIS CERCARIAE

Cort (1914, 1915) was the first worker to describe cercariae from Illinois, and he also described these forms more completely than had been done for any North American larval forms up to that time.

Since then other workers, notably Faust (1918a, 1918b), Miller (1923, 1926a), Beaver (1929), and Horsfall (1930), have published detailed descriptions of one or more Illinois forms. Other references (Faust, 1919; Hopkins, 1933; and Horsfall, 1933) have been made to Illinois larval forms but so far as I am able to determine no other cercariae have been described in enough detail so that they may be included in the following key to the Illinois species.

The present paper includes descriptions of two Gorgoderine Cercariae, six xiphidiocercariae and five furcocercous cercaria, all from Illinois. In addition, a cercariaeum, *Leucochloridium problematicum* Magath 1920, formerly reported from Iowa, has been found in Illinois. This brings the number of described forms from Illinois up to thirty-eight.

C. urbanensis has been found by the author in a new locality, namely Seymour, also *C. trivolvis* at Seymour and St. Joseph. In addition to infecting *Helisoma trivolvis*, *C. trivolvis* was also found in *Physa gyrina*. *L. problematicum* was found at Charleston in an additional species of Succinea, namely *S. ovalis*. Other new localities are Mahomet for *C. gigas*, Seymour for *C. multicellulata*, St. Joseph and Decatur for *C. hamata*, and St. Joseph for *C. wardi*.

A list of the Illinois forms, together with the localities and hosts from which they were reported by various authors, includes the following cercariae.

MONOSTOME CERCARIAE

1. *C. urbanensis* Cort 1914; from Urbana and Seymour; in *Physa gyrina*.
2. *C. robusta* Faust 1918; from DeKalb; in *P. gyrina*.
3. *C. aurita* Faust 1918; from Homer; in *Goniobasis pulchella*.
4. *C. spatula* Faust 1919; from Urbana; in *P. gyrina*.
5. *C. infracaudata* Horsfall 1930; from Homer; in *Goniobasis livescens*.

AMPHISTOME CERCARIAE

1. *C. diastrophia* Cort 1914; from Chicago; in *Helisoma trivolvis*.
2. Cercaria of *Allassostoma parvum* according to Beaver, 1929.
Synonyms according to Beaver:
C. inhabilis Cort 1914; from Urbana; in *H. trivolvis*.
C. convoluta Faust 1919; from Urbana; in *H. trivolvis*.

ECHINOSTOME CERCARIAE

1. *C. trivolvis* Cort 1914; from Urbana, DeKalb, Seymour, and St. Joseph; in *H. trivolvis* and *P. gyrina*.
2. *C. reflexae* Cort 1914; from Chicago; in *Lymnaea reflexa*.
3. *C. chisolenata* Faust 1918; from Mt. Morris; in *P. gyrina*.
4. *C. acanthostoma* Faust 1918; from Urbana; in *H. trivolvis* and *P. gyrina*.
5. *C. complexa* Faust 1919; from Urbana; in *H. trivolvis*.

GYMNOCEPHALOUS CERCARIAE

1. *C. megalura* Cort 1914; from Mahomet; in *Pleurocera elevatum*.

CYSTOCERCOUS CERCARIAE

1. *C. macrostoma* Faust 1918; from Urbana, Homer, and Evanston; in *G. livescens*.

GORGODERINAE CERCARIAE

1. *C. sphaerocerca* E. L. Miller 1935; from St. Joseph; in *Musculium transversum*.
2. *C. mitocerca* E. L. Miller 1935; from Mahomet; in *Actinonaias carinata*.

CERCARIAEA

1. *Leucochloridium problematicum* Magath 1920; from Charleston; in *Succinea ovalis*.

FURCOCERCOUS CERCARIAE

1. *C. douthitti* Cort 1914; from Chicago; in *Lymnaea reflexa*.
2. *C. gigas* Faust 1918; from DeKalb, Mt. Morris, Urbana, and Mahomet; in *H. trivolvis* and *P. gyrina*.
3. *C. minima* Faust 1918; from DeKalb; in *P. gyrina*.
4. *C. robusticauda* Faust 1919; from Urbana; in *P. gyrina*.
5. *C. rhabdocaeca* Faust 1919; from Urbana; in *H. trivolvis*.
6. *C. multicellulata* Miller 1923; from Urbana and Seymour; in *P. gyrina*.
7. *C. hamata* Miller 1923; from Urbana, St. Joseph, and Decatur; in *H. trivolvis*.
8. *C. wardi* Miller 1923; from Urbana and St. Joseph; in *H. trivolvis*.
9. *C. pteractinota* E. L. Miller 1935; from Paris; in *P. gyrina*.

XIPHIDIOCERCARIAE

1. *C. hemilophura* Cort 1914; from Rockford; in *P. gyrina*.
2. *C. isocotylea* Cort 1914; from Urbana and DeKalb; in *H. trivolvis*.
3. *C. polyadena* Cort 1914; from Chicago; in *Lymnaea reflexa*.
4. *C. stilifera* Faust 1918; from Mt. Morris; in *P. gyrina*.
5. *C. trifurcata* Faust 1919; from Urbana; in *P. gyrina*.
6. *C. candelabra* Faust 1919; from Urbana; in *H. trivolvis*.
7. *C. mesotyphla* E. L. Miller 1935; from Muncie, Seymour, Sadorus, Alhambra, St. Joseph, and Rantoul; in *P. gyrina*, *P. gyrina hildrethiana*, and *P. halei*.
8. *C. cystorhysa* E. L. Miller 1935; from Homer; in *Goniobasis livescens*.
9. *C. meniscadena* E. L. Miller 1935; from Mahomet; in *Pleurocera acuta*.
10. *C. acanthocoela* E. L. Miller 1935; from Urbana, St. Joseph, and Seymour; in *H. trivolvis* and *P. gyrina hildrethiana*.
11. *C. steganocoela* E. L. Miller 1935; from Seymour, Sadorus, and Urbana; in *P. gyrina hildrethiana*.
12. *C. pachycystata* E. L. Miller 1935; from Seymour and Henry; in *H. trivolvis*.

The above forms have been arranged in the form of a key for convenience in identification.

Key to the Illinois Cercariae

- 1 (52) (71) Cercariae with tail which is not forked..... 2
- 2 (11) Only one sucker and that at anterior end—Monostome Cercariae..... 3
- 3 (4) Only two pigmented eye-spots—Binoculate
Cercariae.....*C. aurita* Faust 1918.
- 4 (3) Three pigmented eye-spots—Trioculate Cercariae..... 5
- 5 (6) Body spines present.....*C. spatula* Faust 1919.
- 6 (5) Body spines not present..... 7
- 7 (8) Excretory system consisting of only a bladder and two large collecting tubules filled with granules.....*C. urbanensis* Cort 1914.
- 8 (7) Excretory system with accessory capillaries and flame cells..... 9
- 9 (10) Caudal excretory tubule unbranched, except at terminal fork; bladder U-shaped.....*C. infracaudata* Horsfall 1930.
- 10 (9) Caudal excretory tubule with many branches; bladder spherical.....*C. robusta* Faust 1919.
- 11 (2) Body possesses two suckers.....12
- 12 (15) Second sucker ventral and at posterior end of body—Amphistome Cercariae.....13
- 13 (14) Body unpigmented except for narrow area around eyes.....*C. diastrophia* Cort 1914.
- 14 (13) Anterior one-third to one-half of body heavily pigmented.....Cercaria of *Allasostoma parvum* Cort 1914.
- 15 (12) Second sucker ventral but not at posterior end of body.....16
- 16 (21) Body possesses long heavy tail not used in swimming.....17
- 17 (18) Tail has invaginated distal end; not swollen at base—Gymnocephalous Cercariae.....*C. megalura* Cort 1914.
- 18 (17) With heavy tail swollen at proximal end, into which the body may be withdrawn—Gorgoderine Cercariae19
- 19 (20) Distal portion of tail long and filiform; no stylet.....*C. sphaerocerca* E. L. Miller 1935.
- 20 (19) Distal portion of tail not filiform; stylet present.....*C. mitocerca* E. L. Miller 1935.
- 21 (16) Tail slender and used in swimming.....22
- 22 (43) Stylet present.....23
- 23 (27) Tail with fin-fold—Cercariae Ornatae.....24
- 24 (25) (26) Excretory bladder club-shaped.....*C. hemilophura* Cort 1914.
- 25 (24) (26) Excretory bladder small, crenate, with anterior end prolonged into two lateral collecting tubules.....*C. trifurcata* Faust 1919.
- 26 (24) (25) Excretory bladder with two vesicles separated by a very narrow constriction.....*C. mesotyphla* E. L. Miller 1935.
- 27 (23) Tail without fin-fold.....28
- 28 (31) One or two penetration glands on each side—Cercariae Microcotylae....29
- 29 (30) With characteristically wrinkled, rectangular bladder.....*C. cystorhysa* E. L. Miller 1935.
- 30 (29) With U-shaped bladder.....*C. meniscadena* E. L. Miller 1935.
- 31 (28) More than two penetration glands on each side—Cercariae Armatae....32
- 32 (42) Elongated bladder consisting essentially of a posterior vesicle and two lateral ones33
- 33 (34) Many bluish, oil-like droplets throughout body; four penetration glands on each side.....*C. pachycystata* E. L. Miller 1935.
- 34 (33) Body without oil-like droplets.....35
- 35 (38) No trace of digestive ceca posterior to pharynx.....36
- 36 (37) Six to eight penetration glands on each side...*C. isocotylea* Cort 1915.
- 37 (36) Ten to twelve penetration glands on each side...*C. polyadena* Cort 1914.
- 38 (35) With complete digestive tract, including ceca.....39
- 39 (40) (41) Twelve penetration glands on each side...*C. stilifera* Faust 1918.

40 (39) (41) Nine penetration glands on each side.....	<i>C. steganocoela</i> E. L. Miller 1935.	
41 (39) 40) Seven to eight penetration glands on each side.....	<i>C. acanthocoela</i> E. L. Miller 1935.	
42 (32) Bladder composed of anterior and posterior vesicle with medium constriction.....	<i>C. candelabra</i> Faust 1919.	
43 (22) Stylet absent; collar spines developed or absent—Echinostome Cercariae.....		44
44 (49) Swollen excretory crura filled with crystals.....		45
45 (46) Tail with fin-fold.....	<i>C. trivolvris</i> Cort 1914.	
46 (45) Tail without fin-fold.....		47
47 (48) Collar of 40 spines.....	<i>C. chisolena</i> Faust 1918.	
48 (47) Collar of 38 spines.....	<i>C. complexa</i> Faust 1919.	
49 (44) Excretory crura without crystals.....		50
50 (51) Tail with fin-fold; no collar spines.....	<i>C. reflexae</i> Cort 1914.	
51 (50) Tail without fin-fold; collar spines present... <i>C. acanthostoma</i> Faust 1918.		
52 (1) (71) Cercariae with forked tail.....		53
53 (54) Large basal tail stem into which body can be withdrawn—Cystocercous Cercariae.....	<i>C. macrostoma</i> Faust 1918.	
54 (53) Body cannot be withdrawn into tail stem—Furcocercous Cercariae.....		55
55 (60) With no ventral sucker.....		56
56 (57) With two small pigmented eye-spots.....	<i>C. multicellulata</i> Miller 1923.	
57 (56) Without eye-spots.....		58
58 (59) Oral sucker pyriform.....	<i>C. rhabdocaeca</i> Faust 1919.	
59 (58) Oral sucker oval in shape.....	<i>C. hamata</i> Miller 1923.	
60 (55) With ventral sucker.....		61
61 (62) Without eye-spots.....	<i>C. robusticauda</i> Faust 1919.	
62 (61) With two pigmented eye-spots.....		63
63 (64) Furcae longer than one-half the stem length.... <i>C. minima</i> Faust 1918.		
64 (63) Furcae shorter than one-half the stem length.....		65
65 (66) A posterior mucin gland present.....	<i>C. wardi</i> Miller 1923.	
66 (65) No posterior mucin gland present.....		67
67 (68) Four penetration glands on each side of body... <i>C. douthitti</i> Cort 1914.		
68 (67) A great many penetration glands filling the entire region posterior to the eye-spots.....		69
69 (70) Furcal fin-folds closely fluted; without furcal rays.....	<i>C. gigas</i> Faust 1918.	
70 (69) Furcal fin-folds braced with radial furcal rays.....	<i>C. pteractinota</i> E. L. Miller 1935.	
71 (1) (52) Tailless larvae—Cercariae.....	<i>Leucochloridium problematicum</i> Magath 1920.	

V. CYSTOCERCOUS CERCARIAE OF LÜHE

For many years the term Cystocercous was used to designate a miscellaneous group of cercariae, each of which is able to draw its body within the tail appendage where it encysts or remains unencysted. The earliest form to be described for this group is *Cercaria macrocerca* Filippi 1854. All subsequently added members of the group also possessed very large tails and the name Macrocerous was naturally applied to such members. It was found some years ago when their life histories began to be worked out that this grouping was very artificial, and at present three distinct groups should be recognized. Various workers have

spoken of them as the Cystophorous Cercariae, the Cystocercous Cercariae, and the Macrocercous or Gorgoderine Cercariae. I shall consider them in the above order.

CYSTOPHOUS CERCARIAE

History and Definition

Wagener described the first cystophorous cercaria to be reported in 1866, and called it *Cercaria cystophora*. He found it in *Planorbis marginatus*. Sonsino (1892) described *C. capsularis* from *Cleopatra bulmoides* and later Looss (1896) said that it belonged to this group. Pelseneer (1906) reported two marine species of the group, namely *C. appendiculata* from *Natica alderi* and *C. vaullegeardii* from *Trochus cinerarius*.

When Lühe (1909) listed *C. cystophora* in his key to the Cystocercous Cercariae he said about it: "In die Kammer ist ausser dem Körper des späteren Distomums auch das seitlich abgeknickte Schwanzende Zurückziehbar. Entwicklung in Redien in Planorbis."

Sinitsin (1911) defined the group of Cystophorous Cercariae as: "Cercariae which are characterized by possessing a vesicular tail with various appendages upon it." He also added two new marine species.

Cort and Nichols (1920) gave a more detailed discussion of the Cystophorous Cercariae when they added a new species, *C. californiensis*, to the group. They assigned the following characteristics to the group:

1. All cystophorous cercariae except *C. vaullegeardii* develop in rediae.
2. Mother sporocysts where rediae develop have been described for *C. sagittarius* and *C. cystophora*, but none have been found where rediae contain rediae.
3. All species lack larval structures for penetration and encystment; stylet, cystogenous, and cephalic glands absent.
4. Each species has a tail with a central vesicle into which the body can be withdrawn.

Sewell (1922) refers four previously described cystophorous cercariae to his Appendiculata group, along with a new species, *Cercaria indicae* xxxv. His definition of the group can apply to all cystophorous cercariae but he does not explain why he has included only five of the ten forms known at that time in his new group. His definition follows:

- (1) The distome body is somewhat elongate in shape and is colorless and transparent; the two suckers are of equal size.
- (2) The mouth leads back to a pharynx which is followed by a triclad gut.
- (3) The excretory bladder is elongate, reaching forward nearly to the acetabular margin.
- (4) The tail is complex and consists of two parts, a distal, flattened or cylindrical process, and a proximal, rounded or oval, and much swollen portion that forms a cyst containing a long slender filament.
- (5) Development occurs usually in rediae which in turn arise from sporocysts. The redia has a well-marked pharynx and gut, but is devoid of locomotor processes.

C. vaullegeardii and *C. capsularis* differ from the other members of Sewell's Appendiculata group in that they develop in sporocysts instead of in rediae as do the other species.

The following cystophorous cercariae have been described:

- | | |
|---|---|
| 1. <i>Cercaria cystophora</i> Wagener 1866 | 9. <i>C. syringicauda</i> Faust 1922 |
| 2. <i>C. capsularis</i> Sonsino 1892 | 10. <i>C. indicæ</i> xxxv Sewell 1922 |
| 3. <i>C. appendiculata</i> Pelseneer 1906 | 11. <i>C. calliostomæ</i> Dollfus 1923 |
| 4. <i>C. vaullegeardii</i> Pelseneer 1906 | 12. <i>C. invaginata</i> Faust 1924 |
| 5. <i>C. sagittarius</i> Sinitsin 1911 | 13. <i>C. macrocercoïdes</i> Faust 1926 (<i>C. macrura</i> Faust 1921 preoccupied) |
| 6. <i>C. laqueator</i> Sinitsin 1911 | 14. <i>C. biflagellata</i> Faust 1926 |
| 7. <i>C. yoshidæ</i> Yoshida 1917 | 15. <i>C. projecta</i> Willey 1930 |
| 8. <i>C. californiensis</i> Cort & Nichols 1920 | |

CYSTOCERCOUS CERCARIAE

History and Definition

Braun (1891) first described and named one of these anchor-tailed cercariae and gave it the name of *Cercaria mirabilis*. Faust (1918c) added two new species, and described the group as follows:

Aside from their anchor tail the species of this group possess other characters in common which demonstrate their close relationship. Among these are the crowded ceca with granular contents, the long median Y-shaped excretory bladder, the presence of the ovary and pair of testes close behind the acetabulum, and the swollen cirrus pouch anterior to the acetabulum. . . . In general, the cercariae develop as the parthenogenetic offspring of sporocysts. They are found in the respiratory or digestive organs of snails.

Sewell (1922) listed six of the eight forms belonging to this group that had been described up to that time in his new *Mirabilis* group. This *Mirabilis* group, which included only these six cercariae, he described as follows:

- (1) Development probably occurs in sporocysts; but the parthenitæ have only been recorded in three forms.
- (2) The tail is extremely large and wide, and at its anterior end forms a cavity into which the distome may be partially or completely withdrawn, except in the case of *C. fusca*, in which it is connected to the body by a short fold.
- (3) A pair of suckers are present.
- (4) A well-developed pharynx appears to be present, and the alimentary canal consists of a very short oesophagus which almost at once divides into long wide intestinal caeca reaching back to the posterior end.
- (5) The excretory vesicle is elongate and is continued forwards as a median tube to the acetabular region, where it divides into two lateral branches.

The list of described cystocercous forms consists of the following:

- | | |
|---|---------------------------------------|
| 1. <i>Cercaria mirabilis</i> Braun 1891 | 7. <i>C. pekinensis</i> Faust 1921 |
| 2. <i>C. wrighti</i> Ward 1916 | 8. <i>C. stephanocauda</i> Faust 1921 |
| 3. <i>C. anchoroides</i> Ward 1916 | 9. <i>C. splendens</i> Szidat 1932 |
| 4. <i>C. macrostoma</i> Faust 1918 | 10. <i>C. melanophora</i> Smith 1932 |
| 5. <i>C. brookoveri</i> Faust 1918 | 11. <i>C. hodgesiana</i> Smith 1932 |
| 6. <i>C. fusca</i> Pratt 1919 | |

GORGODERINE CERCARIAE

History and Definition

The first cercaria described which belongs in this group is *Cercaria macrocerca* Filippi 1855. Later Wagener (1857) and Thiry (1860) reported this species, but Sinitsin (1905) demonstrated that their reports represented distinct species. Others worked on the life cycles of these forms, but much of the work was not experimentally conclusive.

Lühe (1909) and Sinitsin (1905) agree in calling the *C. macrocerca* of Thiry (1860) a new species. Sinitsin described it (1905) and named it *Cercaria gorgoderae pagenstecheri*. Sinitsin also demonstrated that the *C. macrocerca* of Wagener was a distinct species and named it *Cercaria gorgoderae loossi*. In 1905 Sinitsin described *Cercaria gorgoderae varsoviensis*. The life history discussions which Sinitsin submitted lack experimental evidence.

Kowalewski (1904) described *Cercaria gorgoderae cygnoidis* and Lühe agrees that this is the larva of *Gorgoderia cygnoides*.

The following list includes all gorgoderine cercariae that have been described up to the present time.

1. *Cercaria macrocerca* Filippi 1854
2. *Cercaria gorgoderae cygnoidis* Kowalewski 1904
3. *Cercaria gorgoderae loossi* Sinitsin 1905
4. *Cercaria gorgoderae pagenstecheri* Sinitsin 1905
5. *Cercaria gorgoderae varsoviensis* Sinitsin 1905
6. *Cercaria sphaerocerca* E. L. Miller 1935
7. *Cercaria mitocerca* E. L. Miller 1935

When Lühe (1909) separated these forms from the cystocercous cercariae he described them as follows: "Schwanz ziemlich drehrund und nicht gabelig. Bohrstachel vorhanden. Sporocysten in Sphaerium. (Macrocerke Cercarien von Gorgoderien)."

Sewell divided this group into two divisions according to the relation of the individuals to the adult genera *Gorgoderia* and *Gorgoderina*, but since experimental evidence for such a classification is lacking, I am omitting this subdivision.

During my study of cercariae of the Urbana region, I examined specimens of *Musculium transversa*, taken from the Salt Fork Oxbow just north of St. Joseph, Illinois, on November 24, 1931, and again on February 20, 1932, and found them to be infected with a gorgoderine cercaria. The individuals were found within the gill tissue of the *Musculium*, lying between the gill filaments. After considerable study I found it to be a new species of cercaria which very likely is a larva of one of the Gorgoderinae.

Cercaria sphaerocerca E. L. Miller 1935

(Figs. 1-9)

The distorted appearance of the body and the awkward shape and great size of the tail give to this cercaria a most unusual appearance which is quite distinctive from all other cercariae which I have either studied or noted. The tail is noticeably divided into two distinct regions, a narrow posterior region, greatly elongated, and a swollen or inflated anterior region resembling that of the other gorgoderine cercariae, and several times larger than the body of this cercaria (Fig. 4). At the proximal end of the tail is a bell- or funnel-shaped structure with thickened sides and a central core which serves as an attachment for the body of the cercaria. Just distal to this structure is a constricted, neck-like portion which separates the bell-shaped cyst cavity from the large tail globe. The body is elongate and narrow (Fig. 1), pointed at the posterior end when well extended, and rounded when contracted. The anterior end of the body is narrow but broadly rounded.

The body of this cercaria breaks loose from its tail easily and will do this readily when the cover-slip is pressed, or even because of its own movements in the tap water. I find that the cercariae have broken loose from their tails and are encysted inside the sporocysts after the dead tissues of the *Musculium* have been kept in tap water for about twenty-eight hours. However, they were found also encysted within the cyst cavity or expanded chamber at the proximal end of the tail (Fig. 3), which is quite probably their normal method of procedure. They twist about freely inside the cyst wall, this wall being thin, transparent, pliable and tough, so that it can be pushed out of its normal shape easily. A number of cysts were found in the containers but whether they were cysts of cercariae which had escaped from broken sporocysts, or were formed by cercariae which escaped normally from the sporocysts, is a point which was not determined.

Annular wrinkles extend around the body. Upon examination under high power these wrinkles appear to be horizontal rows of tiny swollen areas which are papillae. These papillae are less distinct on preserved cercariae. Projections are also located on the dorsal lip of the oral sucker. The body is an elongate oval in outline and the ventral sucker is a little posterior to the middle of the body. The posterior portion of the body proper is flattened and appears almost fan-shaped at times.

A small stylet is present, dorsal to the cavity of the oral sucker, but it is somewhat different from that described by earlier workers for other members of this group, as it lacks the lateral points along a ventral sharp blade as well as other structures so well illustrated by Sinitsin (1905),

and Lühe (1909) (Fig. 6). It is connected posteriorly by a group of from six to eight stylet glands on either side of the body just anterior to the acetabulum. These glands connect to a main duct which is bipartite at least in the anterior part of its course.

In the region of the body, posterior to the acetabulum and along either side of the elongated excretory bladder, is located a number of elongate glands in a rather regular group like those pictured for *Cercaria gorgoderæ pagenstecheri* by Sinitsin (1905), but not in as regular a formation as those of *Cercaria gorgoderæ loossi*. When, however, the bladder becomes deflated the regular character of these cells is more likely to be obscured.

The acetabulum is larger than the oral sucker and is posterior to the middle of the body, varying in position from a point just posterior to the center, to the anterior edge of the posterior one-third of the body, depending on the state of contraction since the posterior region of the body in particular is capable of contracting to an astonishing extent. In side view this sucker appears as a cup-shaped structure, attached by its base only to the body of the worm (Fig. 5). No pharynx or prepharynx is present; the esophagus extends posteriorly to a point about two-thirds of the distance from the oral sucker to the acetabulum, before it divides to form the intestinal crura. These crura reach a point slightly posterior to the halfway mark between the acetabulum and the posterior end of the body.

The excretory bladder is a long expulsion canal in this form, reaching from the posterior dorsal excretory pore to a point a short distance posterior to the acetabulum. In side view this bladder is a typical club-shaped structure with its enlarged end forward. Its shape is constantly changing but usually both an anterior and a posterior bulb are evident (Fig. 8). Two main collecting tubules divide into two branches just posterior to the anterior limits of their courses. The anterior branch redivides just posterior to the oral sucker. The posterior branch extends posteriad to a point slightly behind the acetabulum where it divides into two branches, the posterior one of which redivides immediately lateral to the excretory bladder. Finer branches and flame cells are present but their exact location has not been determined.

The following measurements were made while the living worm was subjected to rather strong pressure of the cover-slip, and during a state of contraction, in most cases. When the body is only partially extended it measures 0.576 mm. in length and 0.144 mm. at its greatest width, which is through the region of the acetabulum. The suckers were also measured when the worm was slightly extended and subjected to strong

pressure. The oral sucker is $85\ \mu$ long and $78\ \mu$ wide, while the acetabulum is 0.104 mm. in longitudinal diameter and 0.111 mm. in transverse diameter.

In measuring the tail greater pressure was necessary. The constricted region is 0.616 mm. long and the swollen portion, or globe, is only 0.392 mm. in length by 0.252 mm. in width. Because the greatest width was taken, it was necessary to measure the area just posterior to the center of the swollen part. The bell-like structure in which the cercaria was often found encysted, was measured after the cercaria had broken loose. It is $56\ \mu$ in length and $84\ \mu$ in width when it is contracted. The total length of the entire cercaria is about 1.64 mm.

The large tail is sluggish in its movements but after becoming detached in the water of the container sometimes remains active longer than the body. No definite bodies are discernible in this globe but it is composed of large, clear, irregular-shaped divisions (Fig. 2), which are easily seen when the worm is observed under high power of the microscope. These are similar to the divisions described by Sinitsin (1905) for *C. gorgoderæ varsoviensis*. Rounded nuclear bodies are also located throughout this globe. Smaller angular bodies as well as larger rounded bodies are present but the triangular ones are more common in the posterior portion of the globe (Fig. 7).

In the region between the swollen and constricted portion of the tail, is a small group of tiny glands, which has also been noted in other closely related forms. There are smaller groups in other portions of the tail, with the exception of the central core. The narrow part of the tail, when at rest, possesses peculiar lateral folds which are always located in the same regions of the tail, two groups being on either side of the tail at the base of the constricted portion and two similar groups near the anterior edge of the posterior one-third of this part. It is possible that the muscle fibers of these regions function to produce the swaying, back-and-forth movement, mentioned by Lühe (1909) for similar forms.

This cercaria matures in an elongated irregular sac-like sporocyst which tapers at the end where it is attached to the gill tissue of the host and where there is a prominent knob projecting from the body, although this structure is not always present (Fig. 9). Only brief mention has been given to the sporocyst in descriptions of the few known closely related cercariae. Perhaps one reason for this is that the end opposite the knobbed end is filled with pigment which makes observation difficult. In fact the older sporocysts are not transparent enough to enable one to see the cercariae within, without rupturing the wall of the sporocyst. The young sporocyst extends and contracts in a sluggish manner, but is still as active as some of the cercariae. It is more transparent than those more advanced in age and the germ balls can be seen in

movement quite easily, as they are pushed from side to side during the movements of the sporocyst. The body wall is composed of unusually large cells and becomes thickened at each end of the sporocyst.

When the sporocyst is pressed the cercariae come out of it, and the body of the cercaria, which has been folded at the side of the swollen portion of the tail, when it was in the sporocyst, straightens. Many of the sporocysts contain only one cercaria; perhaps the long period of confinement of the host in the laboratory may have brought about this condition. This again, as has been formerly intimated, might indicate that infection of the molluscan host terminates of its own accord.

Encysted forms were found inside the bell of the tail of individuals in the container. When subjected to pressure of the cover-slip the encysted individual was forced through the opening at the tip of the bell. These recently encysted individuals conform with the description of the cercariae, in their appearance and measurements. Some cercariae have also been found broken loose from their tails and encysted inside the sporocysts that had been freed from the gills of the *Musculium* and left in water over night. Twenty-eight hours after encystment they were still active.

Cercaria mitocerca E. L. Miller 1935

(Figs. 10-15)

The material which furnished the basis for this study was taken from the liver tissue of *Actinonaias carinata* collected in the Sangamon River near Mahomet, Illinois, on Nov. 4, 1932. Very few cercariae from the Unionidae have been reported in North America. This is particularly striking when one considers the abundance of these mollusks in almost all parts of the United States. Altogether about twenty species of these Acephala from various parts of the world have been listed as harboring larval trematodes. From North America, Leidy (1858) reported *Cercaria duplicata* (Von Baer 1827), a rhopalocercous cercaria, and gave as its hosts *Anodonta fluviatilis* and *A. lacustris*.

This cercaria resembles the other gorgoderine cercariae very much, but differs in that it has no stylet. There is no other cercarial group whose members resemble more closely this species, and since it is very similar to these species with the exception which I have noted above, I am including it here.

This cercaria resembles *C. sphaerocerca*, in that it possesses a long tail greatly swollen into a large body at its proximal end, and a narrow distal portion. This posterior portion is very narrow and elongated, being almost threadlike in its appearance (Fig. 12).

The body is large, and elongated when extended, but when mediumly contracted the posterior end appears as a wide flare (Fig. 11) extending

laterally from the sides of the body just posterior to the acetabulum, and much more compressed dorso-ventrally than the rest of the body. The edges of this portion of the body, when the worm contracts, resemble the lobate edge of a contracted turbellarian. The posterior end is quadrate with a constriction in the region of the excretory pore. Lateral constrictions occur on either side of the body just anterior to the ventral sucker (Fig. 10). This feature is evident in nearly all of the cercariae described by Sinitsin (1905) belonging to this group. The surface of the body is broken by several rather prominent papillae which are more common on the anterior end. They extend posteriad along the sides to slightly beyond the posterior limits of the oral sucker.

The size of the body varies greatly according to the state of contraction. When well extended it often is 1.12 mm. in length and 0.269 mm. in greatest width, which in this form is in the region just posterior to the acetabulum, due to the lateral flare already mentioned. When contracted the body is only 0.728 mm. by 0.448 mm., and when only mediumly extended it is 0.952 mm. by 0.336 mm. The portion immediately posterior to the acetabulum often flares outward, out of all proportion to the rest of the body, measuring sometimes as much as 0.504 mm. in width.

The oral sucker is terminal and the mouth opening is ventral. From side view the acetabulum is seen projecting ventrally. The oral sucker is slightly longer than it is wide; it is 0.14 mm. by 0.129 mm. when the worm is only slightly contracted. The acetabulum is located near the middle of the body; it is smaller than the oral sucker and is spherical, being about 0.126 mm. in diameter. It is located about 0.28 mm. posterior to the oral sucker and 0.504 mm. from the posterior end of the body.

The oral sucker opens into an esophagus, no pharynx being present, and extends for approximately one-half the distance from the oral sucker to the acetabulum before dividing to form the two intestinal crura, which are relatively large and which extend to within 0.13 mm. of the posterior end of the body. However, this distance varies greatly with extension and contraction. The esophagus is about 0.104 mm. in length.

No stylet is present, but ducts can be seen running posteriorly from the sides of the oral sucker in a lateral position. The location and number of glands could not be determined in this form, due to the presence of many small cells in this region of the body.

Extending from the posterior end of the body to within a short distance of the acetabulum is the elongated excretory bladder or expulsion canal. When contracted the sides are irregular and the posterior end is swollen into a slight bulb. This bladder and the main collecting tubules resemble greatly those of *C. sphaerocerca*. It divides anteriorly to form the two main collecting tubules which, in the anterior region of the

body, turn posteriorly and run the entire length of the body again. They become indistinct in the maze of tubules, large and small, which ramify the body in all directions. Large flame cells are present in the body.

A mass of large spherical bodies fills the major part of the body posterior to the acetabulum, and when the body is well extended they seem to occupy a definite position and to resemble glands. They are circular but are not systematically arranged, as has been noted in other species (Fig. 11). Also in this area are located three bodies, two of which are lobed, and one unlobed, which I believe to be the reproductive glands. Since the left posterior one is five-lobed and the right anterior is four-lobed, I believe these bodies will give rise to the two testes, one of five parts and the other of four parts, in the adult. For this reason I believe the form may be the larval stage of an adult gorgoderine bladder fluke. The ovary is anterior to the left testis and immediately posterior to the acetabulum.

Like *C. sphacrocerca* this cercaria contains a long, tail-like structure, consisting of an anterior swollen area and a posterior constricted thread-like portion (Fig. 12). The thread contains a hollow core throughout its length, and there are scattered nuclei in both the globe and thread of the tail. The surface of the globe is covered with tiny fissures (Fig. 14), which have no definite arrangement but which connect and diverge in all possible directions.

At the proximal end of the globe is a spherical hollow structure having thickened walls (Fig. 13). The body is attached here, and, when it breaks loose, the opening snaps shut giving the appearance of a closed sphere. The globe measures about 0.448 mm. in length by 0.185 mm. in width, while the narrow portion has the great length of 2.128 mm. and a width near its base of only 22 μ . The width tapers only slightly toward the distal end of this thread.

The sporocysts which give rise to these cercariae are ovate, broadly-rounded sacs, some with contracted tips at the smaller end of the sac (Fig. 15). No independent movement was noted in these forms, even in the younger ones, but the cercariae could be seen within, moving about sluggishly. An average of the measurements of ten taken at random gives a length of 0.980 mm. and a width of 0.462 mm.

A few encysted individuals were found inside the sporocysts in the tissue of the mussel, which had been allowed to remain in water over night after it had been dissected, but there were no tails on these individuals. However, many of these forms remained alive in containers for 48 hours without encysting. Encysted individuals were enclosed by a spherical transparent wall, and they resembled the cercariae in all structural details.

VI. MONOSTOME CERCARIAE

History and Definition

Relatively few of the cercariae described can be placed in this group. Up to the time when Cort (1914) described *Cercaria urbanensis* from Illinois, only two reports of monostome cercariae were available from the literature from North America. Cort (1915) says that they have been known since 1817, but I know of no one of them of which the life history has been proved experimentally. Lühe (1909) described the group as: "Ohne Bauchsaugnapf, mit Augenflecken, Schwanz einfach, lang, schlank, ohne Borstenbesatz. Entwicklung in Redien. Encystierung im Freien (ob bein allen Arten)."

None of these monostomes have been known to develop from sporocysts, but Looss (1896) states that in material of *C. imbricata* near Leipzig he had found rediae in which rediae were developing. A few forms belonging to this group have been described as having posterior locomotor projections on the rediae such as *C. imbricata* Looss 1893. Some of these rediae also bear peculiar constrictions in their bodies, for instance, *Cercaria urbanensis* Cort 1914 and *Cercaria (Glenocercaria) lucania* Leidy 1877.

Sewell (1922) used the term Monostome Cercariae in its broadest sense, to include "all these forms in which as the name implies, a ventral sucker or acetabulum is missing." Since then experimental life history work has been done, notably that by McCoy (1929a) on a lophocercous monostome cercaria, showing that the ventral sucker which has been absent in the cercaria, may develop in the metacercaria. Such discoveries have shown beyond doubt that Sewell's classification of these monostome cercariae is an unnatural one, since many of the forms which he includes in this group are in reality either xiphidiocercariae or furcocercous cercariae. He also divided all monostome cercariae into six subgroups and named them: Pleurolophocerca, Urbanensis, Ephemera, Lophocerca, Lophoides, and Ubiquita. An analysis of this classification shows groups Urbanensis and Ephemera to contain only those forms which had been considered true monostome cercariae up to that time. Sewell's Lophocerca and Lophoides groups contain only furcocercous cercariae while his Ubiquita group contains only stylet cercariae. The individuals of his Pleurolophocerca group differ from those of the Urbanensis and Ephemera groups in that the so-called oral sucker is really a penetrating organ or anterior organ similar to that of the furcocercous cercariae; there are no posterior locomotor pockets; the tail has cuticular fin-folds; and there is no trace of any esophagus or intestinal ceca.

Sewell's *Urbanensis* group corresponds in the main to the binoculate group of Faust (1917) and his *Ephemera* group to the trioculate group of Faust (1917). For the above reasons, I am limiting the monostome cercariae to Sewell's *Urbanensis* and *Ephemera* groups.

I find descriptions of the following species that belong to the Monostome Cercariae:

BINOCULATE CERCARIAE

- | | |
|---------------------------------------|--|
| 1. <i>C. fulvoculata</i> Cawston 1911 | 4. <i>C. hemispheroides</i> Faust 1924 |
| 2. <i>C. konadensis</i> Faust 1917 | 5. <i>C. yenchingensis</i> Faust 1930 |
| 3. <i>C. aurita</i> Faust 1918 | |

TRIOCULATE CERCARIAE

- | | |
|---|--|
| 1. <i>C. ephemera</i> Nitzsch 1807 | 9. <i>C. robusta</i> Faust 1918 |
| 2. <i>C. hyalocauda</i> Haldemann 1842 | 10. <i>C. spatula</i> Faust 1919 |
| 3. <i>C. (Glenocercaria) lucania</i> Leidy 1877 | 11. <i>C. indica</i> XI Sewell 1922 |
| 4. <i>C. imbricata</i> Looss 1893 | 12. <i>C. plana</i> Faust 1922 |
| 5. <i>C. monostomi</i> v. Linst. 1896 | 13. <i>C. trabeculata</i> Faust 1924 |
| 6. <i>C. zostera</i> Sinitsin 1911 | 14. <i>C. infracaudata</i> Horsfall 1930 |
| 7. <i>C. urbanensis</i> Cort 1914 | 15. <i>C. triophthalmia</i> Faust 1930 |
| 8. <i>C. pellucida</i> Faust 1917 | 16. <i>C. lebouri</i> Stunkard 1932 |

Cercaria urbanensis Cort 1914

(Figs. 20-27)

Physa gyrina hildrethiana taken at Camp Creek near Seymour, Illinois, in October, 1931, was found to be infected in unusual numbers with a monostome cercaria, *C. urbanensis*. Study of this form revealed a number of interesting facts which have not been mentioned before.

Table I, which gives the infection records, will indicate that this species is exceptionally numerous in the above-mentioned vicinity. Hosts were collected also in January of 1932, and great masses of rediae were observed in the hepatic tissues. They were elongated sac-like structures (Fig. 26), only 0.263 mm. in length and 0.151 mm. in width in the immature stage, 2.25 mm. by 0.63 mm. in a more advanced stage and 3.42 mm. by 0.936 mm. in the case of large rediae containing developed cercariae.

The almost terminal, muscular pharynx contains noticeable cross-striations in its wall and is spherical in shape. The intestine is large and swollen in most cases (Fig. 24) and the cavity of the body is filled with what appears to be disintegrating tissues and small clear glandular bodies. No living cercariae were seen inside the rediae or in the tissues of the host in the snails collected in January, although, in a few cases, free cercariae were noted in the water after a mass of sporocysts had

been separated from the liver tissue. This may indicate that the length of infection is definitely limited, either by the seasonal factors or by the length of life of the rediae, both of which may result in such disintegration of the rediae in the liver tissue. Many of the rediae are greatly constricted in one, rarely in two areas (Fig. 25), giving the local region the appearance of a neck; also the posterior end, in many cases, is constricted, causing a posterior tail-like structure to be present. The intestine has never been observed extending into this portion.

When the snail hosts are kept in containers at room temperatures the cercariae emerge from them in greatest numbers between 8:00 and 9:00 in the morning. After this period very few leave the snail and in the late afternoon and evening the containers, with scarcely an exception, contain only encysted forms. On successive mornings this swarming is repeated, the snails often being so heavily infected with this form that the walls of the containers are literally covered with these cysts. After swimming about for a very short time they encyst on the glass, or preferably on water plants and sword grass, while the tail beats with great speed, then comes loose and swims about in the water for a long time before finally disintegrating (Fig. 21). The body rounds itself into a flattened circular body and a transparent, stiff covering is formed about it. The cyst is formed so rapidly that it resembles the molting process of some animals, but the substance of the wall is probably cystogenous material. By pressing on the cover-slip this cyst is broken and the worm crawls out. However, I have never observed it to reëncyst.

Almost all of the body is filled with small granules, refractile, and spherical or oblong, which make the cercaria appear almost black under the low power of the microscope. The body is also filled with pigment granules of a brownish or black color which make a detailed study of its structure from the living specimen very difficult if not impossible.

Probably the process of cyst formation is not a continuous one, for after the cyst wall has been formed it can be seen that there are several successive layers (Fig. 22). The body can rotate inside the cyst wall and twists about quite readily (Fig. 23). The following are averages of measurements on five specimens: diameter of cyst 0.232 mm., width of outer wall 28 μ , width of inner wall 28 μ , total width of cyst wall 56 μ .

The tail of the worm is relatively long, as long as the body while resting normally and much longer when extended for swimming. It has a parenchymatous core, and many striations are present which extend parallel to the core.

The body itself need only be characterized here, for its structure has been carefully studied by other workers. Many other very characteristic

features facilitate the identification of this cercaria, namely, the two relatively large, deeply pigmented eye-spots lateral to and almost on a level with the posterior limits of the oral sucker; the central eye-spot, slightly smaller, of a more brownish color, but also heavily pigmented; the condensation of pigment anteriorly and laterally; the elongate oval body, more pointed anteriorly and subquadrate posteriorly (Fig. 20). The narrow esophagus extends only for a very short distance posteriorly before dividing to form the two intestinal ceca which reach almost to the posterior end of the body and extend mesiad to the two large excretory vessels. The small excretory bladder is at the very posterior end of the body. The two excretory siphons are large, swollen, sinuous, and filled with small dark excretory granules (Fig. 20). Posterior locomotor appendages are on the posterior lateral margins of the body on either side of the tail base.

The two siphons which open into the excretory bladder, extend anteriorly on either side of the body, and unite in the region of the oral sucker, in the midline of the body, and just anterior to the median eye-spot. No other part of the excretory system could be distinguished in the body, but the tail contains a central longitudinal tubule which connects anteriorly with the bladder.

It is quite probable that the final host of this cercaria exists in some abundance in this stream, since such a high percentage of infection was found among the snails collected here. Furthermore, muskrats have been noticed several times along the banks and their holes are common in the banks. They are a likely mammalian host for this parasite, and since they are largely herbivores the metacercariae would have an easy method of gaining access into their bodies. This can be confirmed only by further studies.

Encysted forms or metacercariae were found fastened to the shell of the snail as well as on the bottom and sides of the container. The snails were collected on October 26, 1931, and most of the cysts contained living metacercariae on January 29, 1932. The cysts are circular objects, flat on their attached sides and convex on their outer surfaces. The coiled trematode can be seen clearly within (Fig. 23). It is deeply colored by a dark brownish pigment, only the anterior end being light in color. The worm lies in a central cavity and is separated from the cyst wall by a narrow space. The wall of the cyst is composed of layer upon layer of thin, clear homogeneous material. The animal within has elongated greatly and deepened in pigmentation. No trace of the two posterior locomotor appendages can be found, but the large granular excretory bladder is much larger here than in the free swimming stage.

VII. ECHINOSTOME CERCARIAE

History and Definition

In 1909 Lühe placed the Echinostome Cercariae in his Leptocercous Cercariae and characterized this Leptocercous Group as follows:

Distome Cercarien mit ungegabelten und borstenlosem Ruderschwanze, dessen Breite auch im kontrahierten Zustande wesentlich hinter der des Körpers zurückbleibt.

Lühe designated three subgroups of leptocercous cercariae, one of which was the Echinostome Cercariae, and described the latter as follows:

Ohne Bohrstachel. Vorderende mit einem ventral offenen, Kragenartigen Wulst, auf dem ein Stachelkranz zur Ausbildung gelangt.—Schlankschwänzige Cercarien von Distomen mit dem für die Echinostomiden charakteristischen, ventral offenen Kopfkragen, der auch schon bei unreifen, des Stachelkranzes noch entbehrenden Cercarien deutlich kenntlich ist. Reife Cercarien auch bereits mit Stachelkranz. Bohrstachel und Augen fehlen. Entwicklung in Redien, die einen vorderen Ringwulst, zwei hintere seitliche Fortsätze und eine sehr deutliche, dicht hinter dem Ringwulst gelegene Geburtsöffnung besitzen. Encystierung in einem Hilfwirt.

Sewell (1922) devised a rather extensive classification of the Echinostome Cercariae, in which he formed three subgroups: the Echinatoides, the Coronata, and the Echinata. He also included a fourth group of cercariae with the Echinostomes, the Megalurous Cercariae, because his study had convinced him of the relationship of this group to the Echinostomes. However, he does not submit findings which I believe constitute sufficient evidence for such a grouping, so I shall not discuss the megalurous cercariae at this time.

Many of Sewell's characteristics which he applies to the subgroups of Echinostomes can be equally applied to all of these subgroups so that definitive characters of these subgroups are obscured by generalities. Sewell also removed three cercariae from the Echinostome Cercariae since they had been described as having no collar spines, even though it had already been demonstrated that at least two of these individuals developed spines after encystment. I am speaking of *Cercaria agilis* Filippi 1857, *C. reflexae* Cort 1914 and the cercaria of *Himasthla militaris* Van Beneden 1861. He also described a new form without the collar spines, *C. indica* XLI.

Because of the above-mentioned difficulties in a grouping such as that of Sewell, I shall not attempt here to use his groups of Echinostomes. Furthermore, a definite designation of all the described larvae into subgroups seems inadvisable at this time. Several forms that have previously been listed as echinostome larvae must be removed from this group since new families have been created in recent years, such as Acanthostomidae Poche 1926, to include several of the old echinostome genera. Synonymy in the group is common. For instance, the cercaria of

Echinostomum revolutum has been considered by various authors to be *C. echinata* Siebold 1837, *Cercaria A* Tsuchimochi 1926, *Cercaria No. 7* Nakagawa 1915 and *C. limnicola* Faust 1924.

Faust (1924) attempted to divide these cercariae into ten subgroups according to their excretory systems, but such a grouping is impracticable for many forms whose flame cell formulae are unknown. Numerous cystogenous glands prevent accurate determination of the pattern of the excretory system in many echinostome cercariae.

The following forms have been described as possessing no collar spines:

- | | |
|--|---|
| 1. <i>Cercaria agilis</i> Filippi 1857 | 7. <i>C. penthesilia</i> Faust 1921 |
| 2. <i>Cercaria</i> of <i>Himasthla militaris</i>
Van Beneden 1861 | 8. <i>C. indica</i> xli Sewell 1922 |
| 3. <i>C. reflexae</i> Cort 1914 | 9. <i>C. semi-robusta</i> Faust 1924 |
| 4. <i>C. fusiformis</i> O'Roke 1917 | 10. <i>C. pseudo-echinostoma</i> Faust 1924 |
| 5. <i>C. arcuata</i> Cawston 1918 | 11. <i>C. redicystica</i> Tubangui 1928 |
| 6. <i>C. complexa</i> Faust 1919 | 12. <i>C. chitinostoma</i> Faust 1930 |

The following forms do possess collar spines but they are all marine forms:

- | | |
|--|---|
| 1. <i>Cercaria leptosoma</i> Villot 1879 | 4. <i>Cercaria</i> of <i>Echinostomum secundum</i>
Lebour 1912 |
| 2. <i>C. purpurae</i> Lebour 1907 | 5. <i>C. littorinae obtusatae</i> Lebour 1912 |
| 3. <i>C. patellae</i> Lebour 1907 | 6. <i>C. quissetensis</i> Miller & Northup 1926 |

Descriptions of the following fresh-water forms do not include mention of a fin-fold on the tail:

- | | |
|--|--|
| 1. <i>C. echinata</i> v. Siebold 1837 | 18. <i>C. cristacantha</i> Faust 1922 |
| 2. <i>C. echinatoides</i> Filippi 1854 | 19. <i>C. chekiensis</i> Faust 1924 |
| 3. <i>C. coronata</i> Filippi 1855 | 20. <i>C. limnicola</i> Faust 1924 |
| 4. <i>C. spinifera</i> La Valette 1855 | 21. Echinostome <i>Cercaria</i> , <i>Species B</i>
Tsuchimochi 1926 |
| 5. <i>C. number 7</i> Nakagawa 1915 | 22. <i>C. hypoderaei conoidei</i> Mathias 1925 |
| 6. <i>C. catenata</i> Cawston 1917 | 23. <i>C. isidorae</i> Faust 1926 |
| 7. <i>C. trisolinata</i> Faust 1917 | 24. <i>C. equispinosa</i> Brown 1926 |
| 8. <i>C. chisolinata</i> Faust 1918 | 25. <i>C. granulosa</i> Brown 1926 |
| 9. <i>C. acanthostoma</i> Faust 1918 | 26. Echinostome <i>Cercaria</i> , <i>Species A</i>
Tsuchimochi 1926 |
| 10. <i>C. constricta</i> Faust 1919 | 27. <i>Cercaria</i> of <i>Echinoparyphium flexum</i>
McCoy 1928 |
| 11. <i>Cercaria</i> of <i>Echinostomum xenopi</i>
Porter 1920 | 28. <i>C. rebstocki</i> McCoy 1929 |
| 12. <i>C. cucumeriformis</i> Faust 1921 | 29. <i>C. mehrai</i> Faruqui 1930 |
| 13. <i>C. indica</i> xx Sewell 1922 | 30. <i>Cercaria</i> of <i>Euparyphium murinum</i>
Tubangui 1932 |
| 14. <i>C. indica</i> xii Sewell 1922 | 31. <i>C. palustris</i> Chatterji 1933 |
| 15. <i>C. indica</i> xxiii Sewell 1922 | |
| 16. <i>C. indica</i> xlviii Sewell 1922 | |
| 17. <i>C. serpens</i> Faust 1922 | |

Another form, unique along with *C. reflexae* in that it possesses a fin-fold on the tail, was described by Cort in 1914 as *C. trivolvus*. Three large-tailed echinostome cercariae have been described, unlike the other echinostomes in regard to their unique tails. They are *C. magnacauda* O'Roke 1917, *C. caudadena* Faust 1921, and *C. cita* Miller 1929.

Cercaria trivolvis Cort 1914

(Figs. 28-34)

Helisoma trivolvis appears to be particularly heavily infected with this form in Camp Creek near Seymour, Illinois, and in the St. Joseph Oxbow. *Physa gyrina* at St. Joseph, Illinois, was also infected.

The cercaria moves rapidly, either by crawling or creeping on a surface with its suckers or by a rapid lashing of its powerful tail, which is longer than the body when it is extended for swimming. The body is elongated, pointed anteriorly, and broadly rounded posteriorly. When contracted for swimming the body is 0.280 mm. long and the tail is 0.504 mm. long. When only mediumly contracted the body is 0.336 mm. in length. Under pressure it is 0.448 mm. by 0.280 mm. through the acetabulum. When extended the length of the body is 0.549 mm. and of the tail 0.616 mm. At its base the tail is 78 μ wide.

Under slight pressure narrow fin-folds are noted on the posterior half of the tail (Fig. 28). Without careful study it is impossible to see the very fine spines which are located on the anterior portions of the worm. A row of thirty-seven alternating spines partly encircles the body, with a break in the middle of the ventral surface. These spines are quite long, and point posteriad and laterad, except for the angle spines on the ventral surface near the edge of the break, which point toward the midline of the body.

The oral sucker and acetabulum of this distome are relatively far apart, with no very great difference in size. The length of the oral sucker averages about 65 μ and the width 69 μ when the worm is subjected to slight pressure. The acetabulum is 75 μ long and 80 μ wide. Both suckers are almost spherical. The oral sucker is followed by a prepharynx which is almost as long as the pharynx. The pharynx is 33 μ by 31 μ . The esophagus is long and reaches almost to the acetabulum, which is about 0.169 mm. posterior to the oral sucker. The two narrow intestinal crura reach almost to the posterior end. The entire esophagus and intestinal crura are filled with connected masses of tissue which enclose open spaces in what will later be the lumen (Fig. 28). This presents an unusual appearance in the living specimen and indicates that the digestive system is still non-functional. The acetabulum is about 91 μ from the posterior end of the body.

The excretory system is similar to that of most echinostome cercariae. Near the base of the tail on the dorsal side is the excretory pore, and just anterior to it is the small rounded excretory bladder. The two main collecting tubules, or excretory siphons, extend anteriad, and near the level of the anterior margin of the acetabulum they become greatly

swollen with large, uniform, spherical, two-layered, highly refractive excretory granules. These siphons lie intercecally and anterior to the oral sucker, the granules diminishing in number and size anteriorly, and ceasing in the region of the pharynx. The characteristic triangular loop of echinostome cercariae is present, and the tubules turn backward and continue posteriad, in the immediate vicinity of the siphon, almost to the posterior end of the ceca, before dividing into secondary tubules. One secondary tubule extends to the pharynx again before it branches to form capillaries. Flame cells were noted but it is difficult to determine their exact connection with the finer branches of the system because of the great number of characteristically dark cystogenous glands which fill the body except in the regions anterior to the pharynx and immediately anterior to the bladder. Another noticeable feature of this excretory system is the presence of vibratile elements, throughout almost the entire length of the descending siphons, particularly active in the region of the acetabulum and above it part way to the oral sucker.

One tubule extends posteriad into the tail, which soon divides into two lateral branches, each branch extending laterad to the very edge of the tail. Cort (1915) describes these branches as opening to the outside. However, I could detect no sign of an opening here. Perhaps these openings, functional in the early life of the undeveloped cercaria, become closed in the free-living form. Cort states that his material was often secured by dissecting the cercariae from the host tissue, and therefore it is possible that his material differed from emerged cercariae in some respects.

The body contains many large cystogenous glands which fill all available space in the body. Under pressure they can be seen to extend from the oral sucker, to the posterior end of the body, in four rather definite groups or rows. Two rows extend one on either side of the body, lateral to the main excretory tubes; an additional row on each side lies posterior to the pharynx between the esophagus and excretory siphon. They are interrupted by the acetabulum, but, posterior to it, continue to the end of the ceca. The space anterior to the bladder lacks these glands. Each gland is filled with elongated refractile bodies arranged in bundle-like formation.

Groups of glands, perhaps penetration glands, are posterior to the oral sucker, but distinct tubules connecting them with the anterior end of the body were not seen. However, a noticeable feature about this cercaria is a row of six swollen openings along the anterior lip of the oral sucker, each one of which extends posteriorly as a small fiber-like structure. Each fiber is swollen near the dorsal median side of the oral

sucker. Possibly each of these fibers is an undeveloped duct which connects with the penetration glands of the worm.

Masses of cells which are anterior and posterior to the acetabulum, and connected by a narrow row of cells, constitute the genital cell masses.

The tail is much longer than the body when extended for swimming, but under slight pressure is of about the same length. A core extends longitudinally through the tail, and the tissue next to this core contains a row of large, regularly arranged nuclei as shown by an optical section. They are easily seen in the tail when it is subjected to pressure (Fig. 34). A small fin-fold is limited to the distal part of the tail, and I believe this structure has never been described for other echinostome cercariae which have an anterior collar of spines. The posterior end of the tail tapers rapidly for a distance of about 0.114 mm., so that the posterior end is pointed. On some specimens there projects from this distal tip a tiny blunt projection, which is more evident with increased pressure, but which is only rarely seen. Ultra-microscopic projections or granules are present on this structure (Fig. 31) but its exact nature is not known. It is possible that it represents an inverted end of the tail which becomes evaginated under pressure (Fig. 32).

C. trivolvis develops in rediae which are transparent in the young stages but are yellow, and finally brown, in their later stages. The redia is elongated, with narrow posterior and anterior ends, but it varies greatly in shape according to the degree of extension of the body. Near the posterior end of the body are two prominent locomotor appendages, characteristic protrusions extending outward from the body wall and narrow at their distal extremities (Fig. 30). Posterior to these the body is narrowed, ending usually in a small knob-like extension, more prominent when the body is contracted.

Near the anterior end of the body, and a short distance posterior to the sucker, is a broad collar extending around the animal. When contracted, the sucker is partly withdrawn in this collar with only its anterior extremity showing (Fig. 33), but when fully extended a long slender neck separates the collar from the sucker.

Just posterior to the collar lies the lateral birth pore, and, under pressure, the cercariae are slowly expelled from the body through the pore (Fig. 29). Under these conditions the pore is situated on a slight prominence. The anterior opening of the cavity of the sucker is terminal, and posteriorly it opens into a rhabdocoele gut which is about one-half as long as the body. This gut is filled with dark material of a deep reddish-brown color, with the exception of its anterior extremity which is often transparent.

As the redia develops, its body cavity increases in size, even extending into the posterior locomotor appendages, and sometimes contains cercariae and germ balls in various stages of development.

Many of the immature rediae, when contracted, are only 0.347 mm. in length and 0.101 mm. in width, but when extended, measure 0.560 mm. by 62 μ . The mature rediae are about 0.878 mm. by 0.266 mm. but vary from 0.630 mm. to 0.170 mm. in length.

C. trivolvis emerges from its snail host in comparatively large numbers about noon of each day, probably due to temperature increases, but is present in the containers in decreased numbers during the night and early morning.

After swimming about for a time these cercariae enter their snail host again to encyst, or other snails if they are available. It has been found that host-specificity for the encysted cercariae is not very great for I have succeeded in recovering them from laboratory raised *Helisoma trivolvis*, *Physa gyrina*, *Pseudosuccinea*, and *Succinea*.

These cysts are transparent, and the worm coiled within is clearly visible. The spines are more easily discernible but the penetration glands are still indistinct.

During the summer of 1932, encysted forms of this cercaria were fed to a domestic duck which had been raised in a small pen where chances of trematode infection were minimized. Fourteen days after this time, the adult echinostomes were found in the intestine of the duck. Since it was impossible to continue the experiments at that time the experimental feeding was repeated on a more extensive scale during the summer of 1933.

On July 9, 1933, laboratory raised *Physa gyrina* and one *Helisoma trivolvis* were placed in a container with a *Helisoma trivolvis* from St. Joseph which was giving off *C. trivolvis* in large numbers. On July 14 these six snails were examined and a number of encysted forms were found in each. This material was then fed to a three months old duck. On July 28 the duck was examined and twenty-seven large echinostomes were found at the lower end of the alimentary tract attached to the walls of the tract just inside the anus.

Later the experiment was repeated; this time five small *Physa gyrina* and two *Helisoma trivolvis*, all laboratory raised, and previously exposed to emerging cercariae, were fed to a young duck about three and one-half months old. Cysts in one snail were counted and twenty-six were found. After sixteen days mature echinostomes were recovered from the duck.

Further studies are in progress concerning this life history and the identification of the adult, and will be given fully in a later discussion of its life history.

VIII. XIPHIDIOCERCARIAE

Definition

The cercariae commonly termed xiphidiocercariae represent an immense number of forms, differing greatly in their anatomical structure but all having at least one structure in common, namely a stylet. However, it must be borne in mind that other forms not placed in this group also have stylets, for instance, the microcercous and gorgoderine cercariae. Nevertheless, in the xiphidiocercariae this stylet possesses a single sharp point and sometimes a bulb-like swelling, while in other forms the stylet sometimes assumes a complex structure with several points along a ventral border, for example in the gorgoderine cercariae.

Several authors have described the act of penetration into new hosts when the cercaria uses the stylet to make an aperture in the tissue, so that we must conclude that this often functions as a definite penetrating structure.

All members of this large group also possess certain glands, and, as they seem to have no connection with the stylet, I am using the term *penetration gland* which better expresses their true nature. The various formations which these glands may assume, as well as the nature of their usually granular contents, are convenient features for specific diagnosis, and workers have utilized this characteristic even for the designation of subgroups (Sewell, 1922).

Lühe (1909) defined the xiphidiocercariae as follows:

Schlankschwänzige Cercarien von Distomen mit einem Bohrstachel am abgerundeten Vorderende. Augen fehlen. Entwicklung in Sporocysten. Die Encystierung erfolgt in einem Hilfswirt. Hierher besonders zahlreiche und schwer zu unterscheidende Arten.

The xiphidiocercariae have been subsequently divided into a number of groups, the members in each case showing certain structures in common which authors have recognized as showing probable relationship. Lühe (1909) recognized only four such groups: Cercariae Microcotylae, Cercariae Virgulae, Cercariae Ornatae, and Cercariae Armatae. Lebour (1911) placed some forms in a new group, the Spelotrema, and Cort (1915) created another, the Polyadenous Cercariae.

CERCARIAE ORNATAE

History and Definition

Lühe (1909) created the group Cercariae Ornatae to include two species, *Cercaria ornata* La Valette 1855 and *C. prima* Sinitsin 1905, and characterized it as follows: "Distome Cercarien mit Bohrstachel, deren schlanker Ruderschwanz einem Flossensaum besitzt."

Later Cort (1914) added *C. hemilophura* to Lühe's Cercariae Ornatae.

Sewell (1922) created a new subgroup, the Prima group, for his two new species *C. indica* xxiv and *C. indica* xxviii and defined the group as follows:

- (1) Distome Cercariae of moderate size in which the acetabulum is smaller than the oral sucker and is situated behind the middle of the body-length.
- (2) The tail is shorter than the body and is furnished with a dorso-ventral fin-fold in its distal portion: the ventral portion of the fin extends further forwards than the dorsal part.
- (3) The alimentary canal possesses a prepharynx and a pharynx and the intestinal caeca reach back to a point between the posterior margin of the acetabulum and the posterior end of the body.
- (4) Salivary glands are present, and consist of four or five pyriform cells.
- (5) The excretory bladder is oval or rectangular and the main excretory canals are dilated in the posterior part of their course and open into the bladder by a common median orifice. The excretory formula appears to be $2 \times 6 \times 1 = 12$ flame-cells.
- (6) Development occurs in oval or sack-shaped sporocysts.

Faust (1924) made a second subgroup, the Hemilophura, on the basis of the excretory system, and placed *C. hemilophura* Cort 1914 and *C. trifurcata* Faust 1919 in it. He considered both these forms to have the flame cell formula $2 [(3 + 3) + (3 + 3 + 3)]$. However, McCoy (1929) found that *C. hemilophura* does not possess this formula and said it should be removed from the Hemilophura. Thus we have a third subgroup differing from the original two in having an excretory system of the $2 [(3 + 3 + 3) + (3 + 3 + 3)]$ type. To this I shall add one species described below, namely *Cercaria mesotyphla*.

Since the above groups have been based largely on the character of the excretory system, it leaves out of consideration one species of ornate cercaria, *Cercaria racemosa* Faust 1917. Faust did not give the details of the excretory system of this form, but the system is obviously quite different from that of other forms of this group. Therefore, it must be placed in another subgroup. There are naturally many questions about such a classification as has been made for this group of cercariae, but perhaps the above will serve to show the morphological distinctions which various authors have pointed out for different individuals of the Cercariae Ornatae.

At present the group comprises the following:

Subgroup 1. Prima Group Sewell 1922. Subgroup 2. Hemilophura Group

1. *C. ornata* La Valette 1855

Faust 1924.

2. *C. prima* Sinitsin 1905

1. *C. trifurcata* Faust 1919

3. *C. indica* xxiv Sewell 1922

Subgroup 3.

4. *C. indica* xxviii Sewell 1922

1. *C. hemilophura* Cort 1914

5. *C. longistyla* McCoy 1929

2. *C. mesotyphla* E. L. Miller 1935

Subgroup 4.

1. *C. racemosa* Faust 1917

Cercaria mesotyphla E. L. Miller 1935

(Figs. 35-40)

Physa gyrina hildrethiana from the Oxbow at Muncie, from Camp Creek at Seymour, and from East Lake Fork near Sadorus, were infected with *C. mesotyphla*. Also *P. halei* from Alhambra and *P. gyrina* from the Oxbow at St. Joseph and a Pond near Rantoul were parasitized by this form. Infection is quite prevalent in the Urbana region and was particularly high at Rantoul. Hall, in a personal communication, reported the same cercaria from the Embarrass River near Urbana.

In appearance the body is an oval, narrowed in front and more bluntly rounded behind. It is a rapid swimmer and the tail lashes violently from side to side as it propels the body. In regard to its emergence from the host, Hall also states that under constant conditions of light, daylight, and temperature of fifty degrees F., the numbers of cercariae given off are constant, being approximately two hundred over a twenty-four hour period. A decreasing in the amount of light and temperature causes the number of cercariae given off to decrease proportionately.

The body is rather small, being only 0.35 mm. long and 0.308 mm. wide through the acetabulum when contracted. In the same state the tail is 0.308 mm. long. When well extended the body is 0.576 mm. by 0.196 mm. and the tail is 0.616 mm. long, and 0.308 mm. wide near its base.

Spines cover the entire body and are directed posteriorly, being more dense near the anterior end of the body.

The tail is smooth and without a lumen; it possesses a noticeable fin-fold near its posterior half (Fig. 35) which decreases in width near the posterior end. It is attached to the body on its ventral surface, just anterior to the posterior end of the body. It is quite muscular and has longitudinal muscles which are evident when subjected to pressure. There are four groups of these longitudinal muscles which extend in wide spirals through the tail, in addition to a deeper circular layer. It will be seen from the above measurements that the tail is as long as, or slightly longer than, the body of the worm (Fig. 35). The fin-fold extends along the distal two-thirds of the tail.

The acetabulum is slightly posterior to the middle of the body and is smaller than the oral sucker. It is circular and measures 60 μ in diameter, when the body is slightly contracted, while the circular oral sucker measures 84 μ in diameter.

The cavity of the oral sucker opens into a short prepharynx, about 40 μ long, which connects with a thick-walled, muscular pharynx. This structure is 44 μ wide when it is contracted and only 40 μ long. The

esophagus extends posteriad for a distance of 50 to 80 μ and then divides to form the intestinal ceca which reach almost to the posterior end. On the posterior border of the intestinal fork is a noticeable bulge or sac, which persists, to a lesser degree, even when the worm is greatly extended (Fig. 35). This is a characteristic feature of this species.

The stylet points forward and toward the dorsal lip of the oral sucker, and is comparatively small, being only 23 μ in length. It has a clear hyaline base and its walls are thick, particularly in the region of its posterior third, and in the anterior portion just behind the point (Fig. 39).

Lateral to the esophagus, on either side, lies a group of six stylet glands, each of which opens through an individual duct. These glands are particularly indistinct in this species. The main duct which leads anteriad from each group of glands is bipartite in nature. After reaching the region of the stylet it opens above and anterior to the cavity of the oral sucker. These glands do not take neutral red stain as readily as do those of most other forms which I have examined. Many other glands are present in the body, particularly from the acetabulum to the oral sucker and along the sides of the body.

Dorsal to the ventral sucker are two groups of cells, the germ masses. Both masses lie in a diagonal, dorso-ventral plane and their upper ends are narrowed (Fig. 40). This narrowed portion may indicate the beginning of an accessory duct, the oviduct or the vas deferens, since the masses may represent the ovary and testes of the adult.

A body which I believe is a ganglion of the nervous system lies on either side of the pharynx, but the nerves could not be distinguished.

The excretory system is characterized by two spherical bladders, an anterior and a posterior one, which fill and empty in a rhythmic manner, and by a flame cell formula of the 2 [(3 + 3 + 3) + (3 + 3 + 3)] type (Fig. 36). The two alternately contracting bladders, when filled with fluid, are slightly different in size, the upper being about 40 μ wide and 20 μ long, and the lower 30 μ wide by 19 μ long. When the contents of the posterior bladder are expelled through the pore, the anterior bladder empties its contents through the median constriction, the process being repeated rapidly when little or no pressure is exerted on the worm.

The two main collecting tubules proceed anteriad and just anterior to the acetabulum they divide into an anterior and a posterior branch.

The anterior branch on each side gives rise to three branches, one in the region of the cecal bifurcation, one lateral to the esophagus, and one lateral to the pharynx. Each of these branches divides dichotomously into three capillaries, each ending in a flame cell, the location of which can be ascertained by examining the illustration (Fig. 36).

The posterior branch on each side gives rise similarly to three groups

of flame cells, three in each group, its first division being just posterior to the acetabulum, the second opposite the anterior bladder, and the third lateral to the constriction between the two vesicles of the bladder. Of course the locations of the divisions of the tubules vary slightly with contraction, but the locations of the flame cells are constant.

The above flame cell formula shows why I believe this species should be placed in a subgroup of the Cercariae Ornatae along with *C. hemilophura* Cort 1914.

The sporocysts of *C. mesotyphla* are long, slender sacs of a brownish-yellow color, and vary in size. The larger ones measure about 5.70 mm. in length by 0.43 mm. in width and the smaller ones 3.60 by 0.36 mm. Very little liver tissue of the host remained but great tangled masses of sporocysts were present. They were twisted almost inextricably about one another (Fig. 37), each one having one free end while the other end was attached to the central mass. The free end often moves slowly back and forth, resembling some algae in this respect, and even contracts and extends, although not to any appreciable degree.

Each sporocyst contains cercariae and germ balls in various stages of development (Fig. 38). The structure of the immature cercaria is difficult to see because of many small dark cells which crowd its entire body. Those forms having the ability to move about in the sporocyst were very active. No birth pore was noted in the sporocyst.

Dissection of *P. gyrina* from Rantoul, Illinois, disclosed the presence of an infection with cercariae which are identical with the above form in all characteristics which I have noted. However, the sporocysts presented some differences. All five infected snails in this lot contained numerous short sporocysts about 0.592 mm. long by 0.200 mm. wide. This might indicate that these are fairly young sporocysts, even though some of them contained a few mature cercariae.

After living more than thirty hours in tap water these cercariae still failed to encyst. All attempts to make them encyst upon minnows, gold fish, tadpoles, crayfish, and water plants were unsuccessful. I have also attempted to cause their encystment upon isopods, giant water bugs, and whirligig beetles from the regions of infection, but all attempts were unsuccessful. Many dead cercariae were noted in the same containers with these possible intermediate hosts, but no cysts were found.

Because the cercaria is so similar in structure to the adult trematode, *Glypthelmins quieta* Stafford 1900, and particularly because its excretory system is identical to that of *G. quieta* which I illustrated in an earlier publication (1930), I thought that it might prove to be the larval form of this trematode. My findings have shown that both this larva and the adult *G. quieta* occur commonly together in different localities in Illinois.

The only species with which *C. mesotyphla* might be confused is *Cercaria hemilophura* Cort 1914. The fin-fold of *C. mesotyphla* is consistently longer than in Cort's form, *C. hemilophura*; Cort says the whole body of *C. hemilophura* contains small cystogenous glands which fill almost all available space, but I do not find these in *C. mesotyphla*; Cort also said he could not distinguish stylet glands in his species; the bladder of *C. hemilophura* is club-shaped but has two vesicles in *C. mesotyphla*; and no blind pouch between the ceca has been described for *C. hemilophura*. The measurements of *C. hemilophura* differ from those of *C. mesotyphla*.

CERCARIAE MICROCOTYLAE

History and Definition

Sewell (1922) believes these forms represent the most primitive of the xiphidiocercariae. Lühe used the size of these minute forms to separate them in his key, but since it is impossible to place many intergrading forms which have been more recently described, authors have found it necessary to disregard the size element. However, there are other characteristics for the group which aid in a classification of these forms. The great majority of them have no digestive system posterior to the pharynx and the rest have only very short intestinal ceca.

Lühe (1909) listed ten species of microcotylous cercariae and defined the group as follows:

Sehr kleine distome Cercarien mit schlakem ungegabeltem Schwanz und mit Bohrstachel. Körperlänge unter 0.2 mm. Bauchsaugnapf wesentlich kleiner wie der Mundsaugnapf und hinter der Körpermitte gelegen. Stacheldrüsen gering an Zahl (2-4), dicht neben und vor dem Bauchsaugnapf gelegen, häufig von gelblicher, bräunlicher oder grünlicher Farbe. Exkretionsblase klein, mit am verbreiterten Vorderende mehr oder weniger deutlich hervortretender Gabelung. Hautbestachelung bisher nur bei ägyptischen Arten nachgewiesen, aber wahrscheinlich auch bei deutschen Arten vorhanden.

3 deutsche Arten scheinen durch verschiedene Form des Bohrstachels sicher gestellt, einige weitere sind unsicher.

Cort (1915) improved on the above characterization:

1. Developed in gastropods in round or oval sporocysts which are seldom more than twice as long as wide.
2. Cercariae under 0.2 mm. in length.
3. Acetabulum back of the middle of the body and smaller than the oral sucker.
4. Stylet glands not more than four on each side and arranged in rows on each side of the acetabulum.
5. Digestive system undeveloped except for a short prepharynx and a small pharynx.

Sewell, working in 1922, divided these forms into four subgroups, namely the Cellulosa, the Pusilla, the Parapusilla, and the Vesiculosa. However, the separation of these subgroups by Sewell's superficial characters is questionable.

CELLULOSA SUBGROUP

Although Sewell (1922) first used this term and placed in the subgroup *C. cellulosa* Looss 1900 and *C. indica* LVII Sewell 1922, he failed to characterize the subgroup, other than to say: "The possession of only two salivary-gland cells on each side of the body and the simple structure of the excretory system, comprising only four flame cells on each side of the body, appear to me to be sufficient grounds for creating a new sub-group for this form." Probably the following forms also belong to this subgroup: *C. chlorotica* Diesing 1850, *C. brunnea* Ercolani 1850, *C. microcotyla* Filippi 1854, and *C. pseudornata* Lühe 1909.

I have studied three species of microcotylous cercariae which belong to this subgroup:

Cercaria cystorhysa E. L. Miller 1935

(Figs. 41-46)

Collections of *Goniobasis livescens* from the Salt Fork River at Homer, Illinois, in May, 1932, were found to yield large numbers of tiny stylet cercariae, for which I proposed the name *Cercaria cystorhysa* in 1935.

A number of features make this form unique among local cercariae. It is very minute and not easily seen with the naked eye, being only about 0.140 mm. in length even when extended as it crawls along with the aid of its suckers beneath the cover-slip. During such movement the tail is pulled along without beating from side to side—a characteristic of many stylet cercariae—so that its surface is contracted into folds. The tail is not more than one-half of the body in length when they are both at rest and it approximates more nearly a third of the body when they are contracted (Fig. 41).

Ordinarily the body of the worm is about 0.140 mm. by $34\ \mu$ when extended and $73\ \mu$ by $56\ \mu$ when contracted. However, when the extended worm is quieted by the application of pressure it is 0.140 mm. by $50\ \mu$.

The tail is $39\ \mu$ long when contracted and 0.140 mm. when extended, but with pressure it is about $43\ \mu$ long and $23\ \mu$ wide at its base.

The longitudinal diameter of the oral sucker is $30\ \mu$ and the transverse is $27\ \mu$ while the acetabulum is about $18\ \mu$ in diameter. These measurements were taken when the worm was at rest. The acetabulum is located at a point about $33\ \mu$ posterior to the posterior margin of the oral sucker, and it is $39\ \mu$ from the posterior end of the body.

When swimming, the body is contracted and bent ventrally while the tail lashes back and forth in a jerky, spasmodic movement. After

swimming for a time the cercaria settles to the bottom of the container where it remains until death.

The body is elongated and flattened, and the ends are both broadly rounded, with the anterior narrow and the posterior subquadrate. No body spines were noted on this form.

A well-developed oral sucker and acetabulum are present, but the latter is posterior to the middle of the body and noticeably smaller than the oral sucker, being from one-half to two-thirds of it in size. It lies at a point about two-thirds of the body length from the anterior end of the worm.

A central lumen or core is seen in the tail when no pressure is applied, resembling that of other microcotylous cercariae in this respect. Strong pressure and oil immersion enabled me to see numerous tiny hair-like structures on the distal third of the tail (Fig. 44). A few large, deeply-stained nuclei are scattered through the tail.

Located dorsally and in the anterior end of the body is a relatively large stylet about 17 μ in length. It has the characteristic swelling, a swollen circular ridge, at a distance of two-thirds its length from the base of the stylet. The thickened stylet wall does not extend entirely to the base of the stylet, but only a thin membrane surrounds this portion (Fig. 43).

Lateral to the acetabulum on either side of the body is a pair of penetration glands differing from each other in shape and in the nature of their content. The outer gland is more elongate; it consists of three lobes or parts but has only one nucleus, and it takes neutral red stain readily. It is granular but cannot be seen clearly without the application of pressure. The inner gland is more compact, non-lobed, and contains a large spherical nucleus (Fig. 41). The salivary duct, which contains a granular fluid similar in appearance to that of the two median penetration glands, and which is swollen at irregular intervals, passes forward, above the oral sucker. These ducts open, one on either side of the stylet, where granular material exudes, under pressure of the cover-slip. This material adheres to the body surface and remains there as globules for a long time.

Many nuclear bodies are scattered through the outer tissue of the body and stain darkly with neutral red.

Posterior and to the left of the acetabulum is a crescent-shaped mass of tissue which stains a light pink in neutral red; it is probably the primordium of the reproductive system of the adult worm.

The excretory bladder is small and approximately rectangular when not swollen with fluid. This bladder is prolonged laterally where it gives rise to the main collecting tubes of the excretory system. A characteristic

feature of this bladder when only partially filled is its possession of anterior and posterior folds or wrinkles (Fig. 42). During contraction this bladder is rectangular but becomes U-shaped when the body is extended. The folds give a great variety of shapes and sizes to the bladder.

The course of the main collecting tubule on each side becomes lost in a coiled mass of tubules just anterior and lateral to the bladder, but an anterior continuation is present, which soon divides into two branches, the anterior of which extends to the region of the oral sucker (Fig. 42). The minute size of this form made it impossible for me to work out the location of the capillaries and terminal cells.

Cercaria cystorhysa matures in small, oval, or sac-like sporocysts which occur in great masses (Fig. 46) in the liver tissues of *Goniobasis livescens*. They are grayish or semi-transparent in color and are filled with granules and small globules. They vary in shape from spherical to oblong, many possessing knob-like projections at one end, while some are irregular in outline, with constricted centers. An average of eight sets of measurements shows the oblong forms to be 0.195 by 0.121 mm., the oblong forms with knobs to be 0.187 by 0.116 mm., those forms with constricted centers to be 0.174 by 0.101 mm. and the spherical ones to be 0.126 by 0.112 mm.

Mature cercariae move about inside, but in no case were they seen to escape through the walls. No birth pore was noted.

No sporocysts were found which contained more than four cercariae, and I have never seen more than two fully developed ones in a sporocyst (Fig. 45). Only one cercaria was found in many of them, but in such cases the snail hosts had been kept in the laboratory for nearly three weeks before they were examined.

Cercaria meniscadena E. L. Miller 1935

(Figs. 47-51)

The percentage of infection is higher for this species than for any other found in Illinois. Large numbers of *Pleurocera acuta* Raf. were collected in the Sangamon River near Mahomet, Illinois, in October, 1931, and found to be heavily infected with *C. meniscadena*. *Pleurocera* from Oconomowoc River, Wisconsin, was also infected with this cercaria.

The worm crawls with the current under the cover-glass, and moves by extending its anterior end and contracting the posterior end as it is pulled forward. It progresses slowly in this manner, the tail being of no use. When free in the water it has the characteristic, jerky movement of the microcotylous cercariae and moves its body and tail violently.

Later it falls to the bottom of the container, where it rests, congregating there in great numbers.

The body is elongated with a broadly rounded anterior and a narrow posterior end where it is only $18\ \mu$ in width. In the region of the penetration glands and acetabulum the width increases. When well contracted the body is about $89\ \mu$ long by $66\ \mu$ wide. This width was taken through the acetabular region. The tail, at the same time, is $59\ \mu$ in length by $16\ \mu$ in width near its base. When well extended for movement the body is $0.170\ \text{mm.}$ long and the tail $0.150\ \text{mm.}$ When at rest the body is $0.117\ \text{mm.}$ by $52\ \mu$ and the tail is $78\ \mu$ by $17\ \mu$. When under pressure of the cover-slip, and slightly contracted, the body is $0.143\ \text{mm.}$ by $72\ \mu$ and the tail is $0.117\ \text{mm.}$ by $17\ \mu$. When well contracted the body and tail together measure $84\ \mu$ in length, when at rest about $0.124\ \text{mm.}$, and when greatly flattened by pressure $0.224\ \text{mm.}$ It can be seen that the tail is only slightly shorter than the body, whether the cercaria is contracted or extended (Fig. 47).

No spines can be definitely determined for this form; however, under immersion oil, granular-like objects can be noted over the body surface.

The oral sucker is well developed and much larger than the acetabulum, which is a little posterior to the center of the body. The oral sucker is slightly wider than long, being $33\ \mu$ in transverse and $30\ \mu$ in longitudinal diameter. The acetabulum lies about $39\ \mu$ posterior to the oral sucker and is $20\ \mu$ in diameter. It is about $52\ \mu$ from the posterior end of the body. The oral sucker's cavity empties into a tiny pharynx. No prepharynx is present and only a tiny portion of the esophagus was seen. The remainder of the digestive system has never been observed.

The stylet, in the anterior lip of the oral sucker, is $17\ \mu$ long and $5\ \mu$ wide at its base, while the pointed tip at its anterior end distal to the swollen ridge is $5\ \mu$ long (Fig. 47).

Two large penetration glands are on each side of the body, the anterior one being in front of the acetabulum and the other being lateral to it. The anterior gland is darker and more coarsely granular, evidently containing a substance of a different nature. Each gland contains a large clear nucleus. Indistinct bipartite ducts lead forward to the region of the stylet, where they open to the outside. Lying on each side of the body, between the ventral sucker and the two salivary glands, is a transparent body which cannot be seen without great pressure. It presses against the salivary glands, during contraction of the body, and gives to the posterior pair especially, a crescent shape. The transparent body contains a single large nucleus. Perhaps this body is a very early stage of the reproductive glands.

The small excretory bladder is rectangular when not fully expanded,

but when extended it is U-shaped, due to the expanded proximal portions of the two main collecting tubes. Its pore opens to the outside on the dorsal surface. In side view this bladder is seen to have considerable thickness; it measures approximately 26 μ in width and 10 μ in length when expanded.

The two horns of the U-shaped bladder soon narrow into very fine tubules which are so minute and tangled in this cercaria that I was not able to see the connections of their branches with certainty. However, the main collecting tube can be seen to divide in the region of the acetabulum into a posterior and an anterior branch. A division occurs in the anterior branch in the region of the salivary glands. No part of the excretory system could be seen extending into the tail.

The tail has thick walls and a large lumen in its center. Annular constrictions, which are often present even when the animal is greatly extended, characterize it. At times the constrictions are annular, particularly when the tail is greatly contracted (Fig. 48); again they remind one of spirally twisted ridges extending around the tail. During extension these rings disappear at the base of the tail, the smoothing process continuing up to the distal end. About 13 μ from its distal end is a small vesicle which sometimes appears when the worm is subjected to pressure.

C. meniscadena develops in small, swollen, oblong sporocysts which are characteristic of the cercariae of the Cellulosa subgroup. They are massed together in the liver of the host, particularly near its surface.

The ends of the sporocysts are bluntly rounded, so that they have an oval appearance in most cases. The nearly colorless wall is very uneven in its thickness, being very thick in some places, while in others it is very thin but extremely tough (Fig. 49). A central cavity is filled with fluid and contains an abundance of small spherical bodies. Loose strands of tissue are also present in the cavity.

Commonly one, two, and three cercariae are found in the sporocysts, while germ balls are also present (Fig. 50). In many cases disintegrating germ masses are observed. Many sporocysts were also found that contained neither germ balls nor cercariae.

These sporocysts have no power of independent movement, at least in their older stages. Mature cercariae move about for hours, pushing the immature individuals aside in their progress, but none were seen to rupture the sporocyst's wall.

The sporocysts vary greatly in size and shape; however, all are approximately oblong or spherical, many with median swellings or terminal knobs (Fig. 51). The average sporocyst is about 0.185 mm. long and 0.124 mm. wide. Fifteen specimens were taken at random for this

average. The oblong ones average about 0.205 mm. by 0.127 mm. and the more spherical ones 0.154 mm. by 0.120 mm. Two or three cercariae or germ balls were noted in most of the sporocysts but in the majority of cases only one fully developed cercaria was found.

Attempts to induce the free swimming cercariae to encyst in crayfish were not successful. No cercariae were found encysted in the tissue of the snail host.

Cercaria cyclica nov. sp.

(Figs. 52-53)

A number of snails of the species *Viviparus georgiana* at Leesburg, Florida, were examined in October, 1931, for cercarial infection and two were found to be infected with a very small stylet cercaria, for which I propose the name *Cercaria cyclica*. It is the smallest cercaria that I have studied, the body being only 0.126 mm. long when slightly extended and $66\ \mu$ wide through the acetabular region, while the length of the tail is 0.126 mm. When extended for movement the body measures 0.143 mm. by $52\ \mu$ and the tail is 0.124 mm. long and $18\ \mu$ wide near its base.

The worms emerge from the snail in large numbers in the early morning but emergence soon ceases until the following morning when there is another swarming. The body is elongate in shape with a sub-quadrate posterior end, but becomes circular in shape almost immediately when placed on a slide for study (Fig. 52), so that it is impossible to study it in its normal, free-living shape. This circular shape is quite characteristic, for in less than one minute it has rounded into a flattened circle for encystment.

The stylet works incessantly at this time, evidently trying to break through the cover-slip. The tail is lost when encystment begins, and soon it becomes inactive. The worm is an active crawler and makes good use of its suckers. No body spines were seen.

The oral sucker is large, being slightly more than twice the size of the ventral sucker, which is posterior to the middle of the body on a small projection. No part of the digestive system posterior to the cavity of the oral sucker was noted in this form.

The tail is of about the same length as the body; a central cavity extends through its center but no part of the excretory system could be seen in it.

Many small globules are located mainly in two areas of the body, one area being just posterior to the large stylet glands and the other smaller area immediately posterior to the oral sucker.

The worm has an unusually large stylet in proportion to the size of its body, the stylet being $20\ \mu$ long and $5\ \mu$ wide at its base. The swol-

len area or shoulder near its anterior end is also very prominent and well developed (Fig. 52).

Perhaps its most noticeable features are the penetration ducts on each side of the body. In this form there are really two ducts on each side which are not even connected as are the components of the bi- and tripartite ducts of several other forms. The outer, lighter, and finely granular duct opens anterior to the other, and connects posteriorly with a large elongated penetration gland which lies in a transverse position just lateral to the acetabulum. When the body is contracted this gland becomes folded, since its anterior end merges gradually into its duct, giving the appearance of two glands instead of one. The diameter of this duct is not at all uniform, and at one point, about midway in its course, it is swollen into a large, gland-like body, which contains a nucleus similar to that in the gland proper. For this reason two of these glands may be considered to be on each side, instead of only one.

The median duct on each side contains larger droplets of material different from that in the other duct. Anteriorly it ends slightly behind the anterior end of the other duct, and posteriorly it widens into a large body just anterior to the acetabulum (Fig. 52). The two inner ducts both connect to this median enlargement.

A small rectangular bladder, which becomes U-shaped when the body is extended, due to its swollen lateral projections, gives rise to the main collecting tubes. Posterior and lateral to the acetabulum each of these tubes divides into an inner secondary tubule which divides into three branches near the acetabulum, and an outer secondary tubule which divides into a posterior and an anterior branch (Fig. 53). The anterior branch is greatly convoluted anterior to the acetabular level, but finally continues, dividing into three terminal branches lateral to the acetabulum. I believe these are the capillaries of the excretory system but I have never observed flame cells in this form.

After removal of these forms from containers each formed a cyst wall. Tapping on the cover-glass caused this wall to break and the worm to crawl out. However, I have never observed these forms to encyst a second time, for they always died soon after leaving the broken cyst wall.

CERCARIAE ARMATAE

History and Definition

Lühe in 1909 placed a number of xiphidiocercariae in this group because these forms are without a fin-fold and the body length reaches over 0.25 mm. He described the group as follows:

Distome Cercarien mit Bohrstachel, deren schlanker Ruderschwanz keinen Flossensaum und ungefähr dieselbe Länge wie der Körper besitzt. Körperlänge 0.25 mm. überschreitend. Bauchsaugnapt etwas hinter der Körpermitte gelegen und, wenn überhaupt, so doch meist nur wenig an Grösse hinter dem Mundsaugnapt zurückbleibend. Exkretionsblase (soweit bekannt) Y-förmig.

Hierher anscheinend sehr zahlreiche und schwer zu unterscheidende, bisher offenbar auch erst zum kleinen Teil unterschiedene Arten.

Perhaps Lühe's statement that the species of this group are based on small differences, indicates the confusion met with in a study of the classification of these species. He listed ten species as belonging to the group but since then many more have been added.

Cort (1915) created a new group of xiphidiocercariae and gave it the name Polyadenous Cercariae, but Sewell (1922), recognizing the fact that size does not constitute a logical basis for cercarial groupings, considered this a subgroup of the Cercariae Armatae, in spite of the small size of the two forms which Cort placed with the polyadenous cercariae, namely *C. isocotylea* Cort 1914 and *C. polyadena* Cort 1914.

Later Sewell divided the Cercariae Armatae into two subgroups, the Polyadenous Cercariae Cort 1914 and the Daswan subgroup Sewell 1922. Sewell modified Cort's definition of the polyadenous cercariae and gave several characteristics of his Daswan subgroup; but the majority of these characters apply equally to both subgroups. However, the forms which Sewell placed in these subgroups, including his new species, do not follow his own definitions of the subgroups, the result being, so far as I am able to determine, that only two points in these characterizations are sufficiently constant to form a working basis for the assignment of species to them, namely, (1) that referring to the development of the cercariae in elongate sack-shaped or filiform sporocysts in the case of polyadenous cercariae and in sausage-shaped sporocysts in the case of those of the Daswan Subgroup, and (2) the character of the excretory tubules in the two groups.

Even here these two points are highly problematical, for many authors have not described or figured these sporocysts, and secondly, the character of the excretory system in these minute forms is of such a nature that it has never been described in the majority of forms and probably never will be in many of them because of cystogenous and penetration glands which make it impossible to locate already obscure flame cells and capillaries.

Altogether there have been well over fifty of these Cercariae Armatae described from various parts of the world. At least twelve species from the United States have been sufficiently described so that they can be definitely assigned to this group. The list is as follows:

- | | |
|--|---------------------------------------|
| 1. <i>Cercaria polyadena</i> Cort 1914 | 7. <i>C. glandulosa</i> Faust 1917 |
| 2. <i>C. isocotylea</i> Cort 1914 | 8. <i>C. diaphana</i> Faust 1917 |
| 3. <i>C. haskelli</i> O'Roke 1917 | 9. <i>C. dendritica</i> Faust 1917 |
| 4. <i>C. gregaria</i> O'Roke 1917 | 10. <i>C. micropharynx</i> Faust 1917 |
| 5. <i>Cercaria</i> of <i>Lissorthis fairporti</i>
Magath 1917 | 11. <i>C. stilifera</i> Faust 1918 |
| 6. <i>C. crenata</i> Faust 1917 | 12. <i>C. candelabra</i> Faust 1919 |

Several species of *Cercaria Armatae* have been found commonly in the Urbana area, as well as at Leesburg, Florida, and Baton Rouge, Louisiana.

Cercaria acanthocoela E. L. Miller 1935

(Figs. 54-59)

This species was found in *Helisoma trivolvis* from a variety of places, chiefly in the Oxbow at Urbana, the Pollywogs and Oxbow at St. Joseph, and Camp Creek at Seymour, Illinois. It was also found in *P. gyrina hildrethiana* from Camp Creek, Seymour.

It does not remain active for any length of time but soon falls to the bottom of the container where it can be found crawling about among the debris. In swimming, the entire body is twisted from side to side, the tail lashing even more rapidly, the result being an aimless movement, jerky and unsteady, which does not favor rapid movement in any one direction for long. When in motion the body is bent into a loop with the dorsal side outside, and the entire body is somewhat contracted. When it comes in contact with any hard object such as the walls or bottom of the container, it progresses by means of its suckers, and, if a cover-slip is above it, it prefers to crawl with its ventral side uppermost. As it moves in this manner, the contracted tail is dragged along behind the body.

The body is small and oblong in outline (Fig. 55) with its greatest width through the region of the acetabulum when at rest. However, the worm has great powers of extension, extending sometimes until it resembles a long narrow ribbon. When greatly contracted it may be more subquadrate in shape. When subjected to pressure it contracts, the anterior region becoming much wider while the posterior end becomes very narrow. The anterior end is rather bluntly rounded because of the subterminal position of the oral sucker, while the posterior end has pronounced rounded corners because of the presence of setae pockets or caudal pockets on either side of the tail base. When contracted the body is 0.228 mm. in length and 0.111 mm. in width, but when extended it measures 0.355 mm. in length, 85 μ in width. Its length is 0.252 mm. when it is at rest. Neutral red was added to the water to quiet the cercariae and then measurements were taken with little difficulty.

The body is armed with large spines which are situated in rows that run horizontally and diagonally across the body. The spines at the anterior end are quite pronounced but they decrease in size and become more scattered posteriorly, until posterior to the acetabulum they are very small and far apart.

The body wall extends ventrally on either side of the tail, which is attached on the ventral side of the animal near the end of the body. Because of these lateral processes of the body wall, a setae pocket is formed on either side of the tail base, so named because it is open at its anterior end and bears a group of long setae, or spine-like processes, clearly evident from either a dorsal or ventral view (Fig. 54).

The oral sucker is slightly larger than the acetabulum, being $67\ \mu$ in diameter when at rest while the acetabular diameter is $58\ \mu$. The acetabulum is posterior to the middle of the body and, under pressure, or when the body is slightly extended, it is at the anterior edge of the posterior third of the body. It is $91\ \mu$ posterior to the oral sucker and $85\ \mu$ anterior to the posterior end. It does not project from the ventral side of the body.

Tiny papillae are on the ventral sides of both the oral sucker and acetabulum. These papillae are on the inner, ventral edges of the suckers close to the margins of their openings. A short distance posterior to the oral sucker is the pharynx into which a short prepharynx opens. It is of about the same length as the prepharynx, but slightly less in several cases, being only $20\ \mu$ long and $23\ \mu$ wide while the prepharynx is $23\ \mu$ long. Repeated attempts to see the digestive ceca in the living form were unsuccessful until neutral red was added to the water containing the cercariae. This makes them visible when great pressure is applied. About midway between the pharynx and the acetabulum the esophagus divides into two very narrow strands of tissue that represent the future functional crura. For almost their entire course these strands are solid and only in isolated places does a lumen appear. They extend posteriad to a point lateral to the midline of the acetabulum.

The stylet, as is usual, is inserted in the dorsal anterior lip of the oral sucker, and is moved about freely by the movements of the sucker's wall. The anterior third of the stylet is cone-shaped, with its point directed forward and its basal walls thicker than those of the anterior end, so that from a side view it appears to have a thickened hump or shoulder on either side of the base (Fig. 56). The posterior two-thirds is divided into two regions, an anterior part which constitutes by far the major portion of the stylet and which has reinforced walls of the same bluish material that is present in the walls of the cone, and a small posterior portion, darker in color, and solid in texture. It is definitely

separated from the anterior portion and lacks the thickened walls apparent in the rest of the stylet. The stylet is 30 μ long, with its pointed portion or cone not more than 7 μ in length.

A group of penetration glands lie lateral and anterior to the acetabulum, on either side of the body, the median glands of each group extending mesiad so that sometimes they meet in the region immediately anterior to the acetabulum. Repeated efforts to count the glands on each side gave varying results and led to confusion until it was finally determined with certainty that the same number of glands was not always present. This is interesting in that it is contrary to the condition in many other cercariae with regard to this character. It is often regarded as a character of great specific value in the identification of species but it is to be noted here that we must make allowance for this variation. Pressure enables one to see all of the glands clearly, each with its own nucleus, since they are all brought to the same level. Seven or eight glands are located on each side—only rarely are there six or nine—the median ones meeting in the midline.

A pair of ducts, two on each side and in close proximity, extend antieriad to the side of the stylet where they open to the exterior. The two ducts on each side twist about each other. This, together with the fact that they possess numerous swollen areas throughout their lengths, differentiates them from many other forms similar to *C. acanthocoela*.

Many small glands fill the body, both anterior and posterior to the acetabulum, each with a dark nucleus when in neutral red. A compact group of small cells lies between the oral sucker and the pharynx. Lateral bodies are thought to be cystogenous glands.

Two cell masses are present which are the early stages of the reproductive organs. One lies just posterior to the acetabulum and the other is just anterior to it. They are connected by a narrow strand of cells extending postero-laterad around the acetabulum.

Normally the excretory bladder, because of a constriction in its anterior region, consists of a posterior or caudal vesicle which opens anteriorly into a narrow lumen, and two lateral vesicles or arms with which this lumen connects. The walls of these arms, or bases of the main collecting tubules, are flexible so that they become periodically swollen with fluids (Fig. 54). This gives the appearance of three bladders in this cercaria, an important characteristic in identifying it if the functioning of the various parts is understood. The connections between these parts of the bladder are difficult to see when no pressure is applied (Fig. 57). Periodically the two lateral vesicles connect so that at this time there appears to be a single bladder, which extends horizontally. The fluids are then forced toward the center so that a median anterior vesicle

is formed, resembling greatly the above-mentioned caudal vesicle. The fluids are emptied posteriorly and fill up the caudal vesicle from which they are emptied to the outside through the excretory pore. This lies on the dorsal surface near the base of the tail, a short distance anterior to the posterior edge of the body. Under strong pressure the connections between these bladders all become widened so that the bladder is broad throughout its entire length and resembles a large Y. When the median part is sharply constricted the bladder appears to consist of an anterior V and a posterior rectangle. Thus the bladder assumes a great variety of shapes during its functioning and with varying degrees of pressure exerted by the cover-slip.

A main collecting tube extends antieriad from each arm of the bladder, and divides lateral to the acetabulum into a posterior and an anterior secondary tubule. The anterior tubule has four branches, and the posterior one two branches, each branch ending in three capillaries. Thus there are eighteen capillaries in each side of the body, twelve anterior and lateral to the acetabulum and six posterior to it. Of the anterior branches, one is lateral and slightly anterior to the acetabulum, one is lateral to the esophagus, one is lateral to the pharynx, and the fourth is immediately posterior to the oral sucker. The anterior branches of the two posterior ones are lateral to the median constriction of the bladder, and the posterior branch is in the extreme caudal portion of the body lateral to the setae pocket. Only four flame cells were identified but no doubt each capillary ends in one of these terminal cells.

The tail is usually contracted as it is pulled along by the animal. Since it has great powers of extension, large folds occur along its sides when contracted, but no fin-fold is present. When free from pressure, a lumen or core can be seen extending through the center. Scattered nuclei are present in the tail, an average of fourteen being there for four specimens studied. The width near the base of the tail is $36\ \mu$. When contracted it is 0.117 mm. long and when mediumly extended it is 0.247 mm. long.

Nearly all of the liver tissue of the snail host had disappeared and the sporocysts were present in great tangled masses. These sporocysts are elongate, sac-like structures (Fig. 58) and vary a great deal in size and shape, depending on their age. The length varies from about 0.2 to 0.78 mm. and the width from $15\ \mu$ to 0.24 mm. The walls are thin and prolonged into knobs and swellings at various points. Cercariae, when mature, travel rapidly from one end of the sporocyst to the other and occasionally break through a tiny opening at one end of the sac which they have made by their own efforts. They squeeze slowly through

this opening and swim actively away. At least in the older stages these sporocysts have no power of independent movement.

Immature cercariae in a single sporocyst range from ten in number to one hundred and fifty.

In one large reddish-brown pigmented sporocyst a large quiescent cercaria was observed. It acted much as an encysted individual, had no stylet, was as large as, or larger than, the metacercaria of this form and resembled the metacercaria in anatomical respects, but had a small undersized tail which moved only slightly. This cercaria was nearly as wide as the sporocyst and appeared to be a giant individual among its fellows. Whether it represents a dual infection of the snail or a phenomenon as yet unexplained in the life history of *Cercaria acanthocoela* remains to be solved. I believe that this represents a cercaria that for some reason has failed to encyst.

Encysted individuals were taken from cysts in the liver tissue of the snail host (Fig. 59), but their scarcity indicates that in the majority of cases the cercariae do not return to encyst there.

When the transparent cyst wall was broken the worm moved about freely. The intestinal ceca are readily visible, reaching nearly to the posterior end of the acetabulum, and are noticeably wider and more developed than in the cercaria. No stylet was present but it was noticed in the wall of the cyst before the cyst was broken. The bladder is filled with granules and is truly Y-shaped. Spines are about evenly distributed over the entire surface of the body, and can be easily seen now. The body is about 0.280 mm. by 0.123 mm. and the distance between the oral sucker and the acetabulum is 61 μ . The acetabulum is 52 μ in diameter and 81 μ anterior to the posterior end of the body. The oral sucker is 69 μ wide and only 57 μ long.

Attempts to recover encysted individuals of this form from several species of fish, crayfish, and a number of frogs, were unsuccessful. However, cysts were found on *Notropis whipplei* Girard, sixteen hours after cercariae had been placed in the dish containing the fish. These cysts were fed to turtles, but with negative results.

C. acanthocoela differs from similar forms in several respects. It cannot be confused with *C. isocotylea* Cort 1914 because of the undeveloped digestive tract in *C. isocotylea* as shown by Cort (1914) and Faust (1918a). *C. polyadena* Cort 1914 has no esophagus or digestive ceca and has from ten to twelve penetration glands. *C. glandulosa* Faust 1917 differs radically from this form in the size of its stylet, the character of its excretory system, and the possession of numerous glands about its digestive tract. *C. diaphana* Faust 1917 differs from *C. acanthocoela* in the nature of its anterior glands, the proportions of its

two suckers, and the nature of its excretory system. Finally, *C. indica* XVII Sewell 1922 differs markedly from *C. acanthocoela* in regard to its excretory system, the size of its stylet, and its genital cell masses.

Cercaria tricystica E. L. Miller 1935

(Figs. 60-65)

This xiphidiocercaria was found in *Helisoma lantum* at Baton Rouge, Louisiana. Collections were made in June, 1932.

The body is bent ventrally and the tail lashes violently as the worm moves through the water. Soon the cercariae sink to the bottom of the container where they congregate in large numbers.

The body is flattened dorso-ventrally, much like that of *Cercaria mesotyphla*, and is oval in outline. When contracted or at rest the body is widest just anterior to the acetabulum. When at rest it is 0.224 mm. by 78 μ , when well extended 0.308 mm. by 56 μ and when contracted 0.196 mm. by 0.112 mm. The greatest width of the body when contracted is about 0.124 mm.

Spines are present at the anterior end of the body but they become smaller and more scattered posteriorly, until, posterior to the acetabulum, they cannot be seen.

The suckers are disproportionate in size, the oral sucker being larger than the acetabulum. It is about 52 μ in length and 59 μ in width when at rest, while the acetabulum is only 39 μ in diameter. The acetabulum is posterior to the middle of the body, being about 65 μ posterior to the oral sucker and 59 μ anterior to the posterior end of the body. It is located at the division of the median and posterior thirds of the body. The ventral sucker does not project ventrally as is common in many of these forms.

A short prepharynx and relatively large pharynx lead from the oral sucker. This pharynx is about 20 μ long and 20 μ wide. Posterior to it extends the narrow esophagus, which soon divides to form the equally narrow crura. They extend posteriad to about the horizontal diameter of the acetabulum (Fig. 60). These crura contain very narrow lumens, with numerous cell-masses, indicating that they are still undeveloped.

The mucin glands constitute a noticeable and distinctive feature of the cercaria (Fig. 61). Each lateral group is composed of nine glands which lie in the dorsal region of the body and lateral to the acetabulum. Each gland contains a large clear nucleus. All of the glands on each side connect anteriorly with a large lateral duct. In its posterior region this duct is bipartite but near the oral sucker each duct is composed of four separate parts (Fig. 60). Each duct opens at the side of the stylet, anterior to the walls of the oral sucker. Under pressure a sticky material passes out through the openings of these ducts.

The stylet is about $21\ \mu$ long and contains a dorsal ridge or swelling near the base of its anterior third (Fig. 62). A side view of the stylet indicates that this ridge does not extend ventrad (Fig. 63.)

A genital mass, which stains pink in neutral red, lies posterior to the acetabulum and extends to the right, passing forward along the right side of the acetabulum. It is composed of small spherical cells.

The excretory bladder varies in its shape because of pressure and contraction of the excretory pore. However, when not distended with fluid it often consists of three vesicles, because of a median constriction which separates the two lateral arms from the posterior vesicle. This constriction is weaker at times so that a broad T is formed. When the body extends this shape changes to a Y. The constriction is so minute that three separate bladders seem to be present. In this respect it resembles *C. acanthocoela*.

A main collecting tubule extends anteriad and laterad from each lateral vesicle and, lateral to the ventral sucker, divides into an anterior and a posterior secondary collecting tubule. The connections of the finer branches of this system have not been determined.

The tail is attached ventrally as noted above, and when the worm is not swimming the tail is contracted and has an irregular outline. It is much shorter than the body, being only $84\ \mu$ long when contracted and $34\ \mu$ wide at its base. When slightly extended it is 0.140 mm. long and when well extended it is 0.174 mm. long. Very narrow caudal pockets are present because of the above mentioned tail attachment but they are not readily seen. No setae are present in these pockets as is true of the pockets of *C. acanthocoela*. No caudal excretory tubule is visible but a narrow lumen is present in the tail which can be observed without the application of pressure. The tail has no fin-fold (Fig. 64).

C. tricystica develops in the liver tissue of *Helisoma lantum* which is crowded with very small oblong sporocysts, similar to those of *C. meniscadena*. In old infections I have never found more than one fully developed cercaria in a sporocyst (Fig. 65), but in the young forms several fully grown as well as numerous developing cercariae were found. The sporocysts possess transparent walls, but the walls of those containing active cercariae may contain a light golden-brown pigment. The wall is elastic and easily distorted by the cercariae within. It is thin and composed of large cells joined to each other in an irregular formation. Large individuals were 0.273 mm. long and 0.137 mm. wide but the averages for ten individuals were 0.195 mm. in length and $85\ \mu$ in width.

Cercaria tricystica differs from both *C. isocotylea* and *C. polyadena* in the relatively small size of its tail, the shape and size of its sporocysts,

the number of salivary glands, the possession of a complete digestive tract, and in several characteristics of less importance. It is unlike *C. diaphana* in that *C. diaphana* has caudal pockets lined with setae, a stylet nearly twice as large as that of *C. tricystica*, and eight penetration glands of much smaller size. The only other forms at all like *C. tricystica* are *C. glandulosa* and *C. indica* XVII. *C. glandulosa* contains numerous glands which line the digestive system; it also has pronounced caudal pockets which have spines as does *C. diaphana*, and it has a stylet larger than that of *C. tricystica*. *C. indica* XVII has pyriform salivary glands which differ in number and in the character of their ducts from those of *C. tricystica*.

Cercaria cystonchnoides E. L. Miller 1935

(Figs. 66-70)

Ampullaria depressa, collected at Leesburg, Florida, in October, 1931, was found to be infected with a very large stylet cercaria. The cercariae and sporocysts were present in the liver in immense numbers, such swarms of cercariae emerging that they soon made the bottom of the container white, when they began crawling about with their suckers. They continue their movements long after the tail has been lost. They remained alive in the containers for a period of thirty-six hours.

The body is elongate, bluntly rounded at its posterior end and drawn out to form a narrow projection at its anterior end (Fig. 68). A depression occurs at the posterior end of the body in which the tail is attached. The body is about 0.616 mm. long and 0.325 mm. wide when at rest. This width was taken through the acetabulum. The tail is about 0.504 mm. long when at rest. Thickly-set spines cover the entire body surface and are larger in the anterior region.

The large stylet is about 36 μ long and bears a swollen area, or ridge, at the base of its pointed anterior end (Fig. 67).

The tail, which has almost the same length as the body, has no excretory tubule, but its longitudinal core disappears when the tail is fully extended (Fig. 66). It possesses no fin-fold.

Numerous glands make obscure the excretory tubules in this form and many tiny globules are present through the entire body.

The penetration glands lie anterior to and on either side of the acetabulum, each lateral group consisting of a large number of glands so that I was not able to determine the number. The large penetration duct can be seen extending along either side of the body to its termination at the anterior end of the body near the base of the stylet. This termination is swollen, the enlargement being particularly noticeable when the worm is resting or is contracted.

The oral sucker occupies an antero-ventral position, being not quite subterminal. It is circular, being about 0.101 mm. in diameter. The acetabulum occupies a position very near the center of the longitudinal axis of the body but slightly posterior to it. It is unique in being slightly larger than the oral sucker, for it is about 0.104 mm. in diameter. The body extends 18 μ anterior to the oral sucker and 0.182 mm. posterior to the acetabulum. The ventral sucker is 74 μ posterior to the oral sucker.

The cavity of the oral sucker, opening posteriorly into a short prepharynx which is continued posteriorly into a muscular pharynx, measures about 0.299 mm. in length and 0.351 mm. in width when quiescent. The relatively short esophagus divides to form the intestinal crura which extend posteriad as narrow lateral tubes, reaching almost to the posterior end of the excretory bladder (Fig. 69).

The large excretory bladder opens through a dorsal pore to the exterior. It is rectangular when in a contracted condition, but when filled, it extends anteriorly as a large cone-shaped or pyriform vesicle, reaching to within a short distance from the acetabulum, where it divides into two antero-lateral siphons.

A mass of cells which probably represents the genital primordium lies posterior to the acetabulum.

Each siphon crosses the cecum and divides just anterior to the acetabulum, into an anterior and a posterior secondary tubule (Fig. 69). The posterior branch divides ultimately into three branches, each branch of which ends in three capillaries, each with its flame cell. The flame cells, which are constant in position, total thirty-six, eighteen on each side of the body. The anterior secondary tubule divides similarly into nine such collecting tubules. The anterior group of flame cells lies lateral to the oral sucker, prepharynx, and pharynx; the second group is in the immediate region of the union of the intestinal ceca; the third group is in the region between the second group and the acetabulum; the fourth group is lateral and posterior to the acetabulum; the fifth group is lateral to the excretory bladder; and the sixth group is lateral and posterior to the excretory bladder.

Upon opening this infected *Ampullaria depressa*, I found the liver to be bulging with thousands of cercariae and sporocysts in all stages of development. The excretory tubules and the bladder were not seen in the very young cercariae.

These sporocysts were quite heavily pigmented with a brownish-red pigment. Slight movement could be noted at the knob-like ends but the swollen portions, at least in the older individuals, exhibited none whatever. The majority of the sac-like sporocysts have two swollen areas filled with cercariae, and a neck-like constriction between them (Fig. 70). In many of the younger sporocysts only one of these swollen

areas is present. Matured cercariae are actively engaged in striking against the walls of the sporocysts with the stylet, and occasionally one escapes through the opening made in this wall. Germ balls in various stages of development are also present in large numbers.

This species represents one of the few forms ever described from Florida, and no Cercariae Armatae have ever been described which might be confused with it because of close similarity in structure. Its large acetabulum sets it apart from most other related forms. A few of these cercariae have been described which also have a larger ventral sucker than oral, such as *Cercaria tenuispina* Lühe 1909 and *Cercaria triloba* Filippi 1857, but confusion can be avoided because of other differences between these forms.

Cercaria steganocoela E. L. Miller 1935

(Figs. 71-73)

The host of this species of cercaria is *Physa gyrina hildrethiana* which was collected at Camp Creek near Seymour, East Lake Fork near Sadorus, and in the Drainage Ditch at Urbana. Material was obtained in April and May, 1932. Scarcity of material gave me no opportunity to make detailed studies of several features.

This form resembles *C. acanthocoela* and casual observation gives one the impression that it is *C. acanthocoela*. However, several differences will be mentioned.

It is an elongated stylet cercaria, having the jerky movement characteristic of these forms, as it swims through the water.

The body is narrower near its posterior end, and a little anterior to the posterior margin on the ventral surface of the body is the attachment of the tail. A deep groove on this surface into which the tail fits, gives rise on either side of the tail base to a deep caudal pocket which has its lateral wall lined with small spines, directed inward (Fig. 71).

Many spines are on the anterior end of the worm. They become smaller and are more scattered posteriorly until, in the region of the acetabulum, they are not visible.

The body and tail are of about the same length. With only little pressure the body is 0.224 mm. long and 0.168 mm. wide when contracted. When at rest both the body and tail are about 0.280 mm. in length, and the tail is 45 μ wide at its base. However, when well extended the body may be 0.460 mm. by 80 μ . The tail is pulled along as the cercaria crawls, beating vigorously when body movement occasionally ceases. A lumen is visible when the tail is not compressed. No fin-fold has been seen on the tail.

The penetration glands are peculiar in that in addition to a group of five to six glands on either side of the acetabulum, there are a few small

glands attached to the side of the ducts (Fig. 71). Two small glands are usually thus attached on each side but their position is so indistinct that it is impossible to determine their number with certainty. The large duct on each side of the body is twisted and swollen at intervals. It is narrower near the posterior border of the oral sucker, then swells and from here on to its anterior opening is tripartite. Just before opening to the exterior on either side of the stylet the ducts narrow rapidly and become very fine tubules, still, however, retaining a median striation. Droplets of a thick consistency form at the openings of these ducts when pressure is applied to the animal. Other small glands cover the body.

Small flattened masses of cells which represent the undeveloped genital system lie anterior and posterior to the acetabulum (Fig. 71).

The oral and ventral suckers are approximately of the same size. The length of the oral sucker averages $69\ \mu$ and its width $66\ \mu$, while the ventral sucker is $67\ \mu$ by $65\ \mu$. When the worm is at rest these two suckers are spherical. The acetabulum is in the center of the body, at a distance of $70\ \mu$ from the anterior end and $0.104\ \text{mm.}$ from the posterior end of the body. It is $98\ \mu$ behind the oral sucker.

The stylet contains the characteristic thickening of cercariae armatae near the base of its anterior third (Fig. 72). This thickening does not extend ventrally as determined from side views. Near its base the thickened walls cease at a circular constriction and the stylet ends in a globular base which is enclosed in a thin wall and is composed of a homogeneous hyaline substance. The stylet averages about $27\ \mu$ in length but occasionally reaches a length of $30\ \mu$.

Posterior to the oral sucker is a very short prepharynx which sometimes does not show. Posterior to it is the pharynx, an esophagus, and finally the two intestinal crura, which extend to within a short distance of the posterior end, almost to the back margin of the posterior excretory vesicle if the worm is not fully extended. These crura are very narrow and rudimentary and can seldom, if ever, be seen in the living animal unless it is stained with neutral red or a similar stain; then the worm must be subjected to great pressure. The tissue inside the walls of the crura stains deeply with neutral red and this material is seen to fill the lumen only at irregular intervals (Fig. 71). The development of this digestive system is an important difference between this form and *C. acanthocoela*, and constitutes one of the few useful characters in a separation of the two species.

The expulsion canal is composed of the following elements: a posterior rectangular vesicle which connects with its excretory pore by a narrow, short tube that opens at the base of the tail on its dorsal side; a median

anterior constriction which extends forward for a short distance before dividing to form a Y; and, finally, two lateral elongated vesicles posterior and lateral to the acetabulum which are really expanded portions of the basal ends of the main collecting tubules. These bladders often communicate with each other by forming a broad, transverse, single vesicle just before emptying into the posterior vesicle through the constricted anterior end of the median expulsion canal. Again, all three may be broadly joined so as to make a typical Y-shaped bladder. The bladder functions much as it does in *C. acanthocoela*. Main collecting tubules extend anteriorly from these lateral bladders but the connections of the finer tubules and capillaries of the excretory system have not been determined.

Large, elongate, irregularly-shaped, yellowish sporocysts are found in the liver tissue of the host (Fig. 73). They are filled with many cercariae in different stages of development, from undifferentiated germ balls to mature cercariae seeking a way out of the sporocyst. Older infections were noted in which the liver seemed to be disintegrating even though the snail was still alive. In these snails the tissue was soft and broken but still filled with active cercariae. Many old, partially disintegrated sporocysts were found in these snails. It may be that when the cercaria is parasitic in several different species of snails, the more uncommon hosts are not able to withstand heavy infections, so that there is an actual disintegration of the liver, with the consequent death of the sporocysts. Disintegrating germ balls were also present. The average dimensions for ten of these sporocysts were a length of 4.5 mm. and a width of 0.252 mm.

While the digestive system of *C. steganocoela* is similar to that of about fourteen other species of these Cercariae Armatae, other differences such as the number of salivary glands on a side, the shape of the excretory bladder, the presence of large caudal glands, and the character of the genital masses enable me to differentiate it readily from these forms. I refer to such forms as the following: *C. stylobuccalis* Faust 1922, *C. microxiphifera* Faust 1926, *C. paracephaladena* Faust 1926, *C. cawstoni* Faust 1919, *C. ingrabilis* Faust 1921, *C. humilis* Faust 1921, *C. cephaladena* Faust 1921, *C. tenuispina* Lühe 1909, *C. triloba* Filippi 1857, *C. limnaeae ovatae* v. Linstow 1884, *C. secunda* Sinitsin 1905, *C. dimorpha* Sinitsin 1911, and *C. cribrata* Sinitsin 1911. *C. candelabra* Faust 1919, the only North American form described up to this time which resembles *C. steganocoela* most in regard to the digestive system, differs from it in having an excretory bladder very different in shape, a smaller oral sucker, acetabulum and stylet, and in having a cluster of penetration glands on each side of the body with many in each cluster, while there are only from five to seven on a side in *C. steganocoela*. *C. pseudarmata*

Brown 1926 has only four penetration glands on each side, and *C. leptosoma* Brown 1926 has five.

It should be noted here that synonymy may be the result of earlier descriptions of forms in which no digestive system was seen beyond the pharynx, for improved methods have probably enabled workers to see structures which were not seen by earlier workers. This has been discussed earlier in this paper.

Cercaria pachycystata E. L. Miller 1935*

(Figs. 74-77)

Helisoma trivolvis collected at Camp Creek near Seymour, Illinois, in October, 1931, and at Mud Slough near Henry, Illinois, in June, 1932, was found to be infected with a small xiphidiocercaria belonging to the Cercariae Armatae. A detailed study in 1935 convinced me that this was a new species of cercaria.

Measurements were difficult to make on this worm since it was continually in motion, until the pressure of the cover-slip was great enough to distort the worm, or until stain such as neutral red or methylene blue had been added to the water. It crawls aimlessly about; the contracted tail is pulled along, and at intermittent periods it is extended and beats rapidly from side to side, as occurs commonly in the Cercariae Armatae.

The body is usually an elongate, flattened oval, but ribbon-like when well extended. The anterior end is narrower than the posterior end, which is broad and truncate. The posterior end is elevated dorsally so as to make a hump above the ventral attachment of the tail. The small acetabulum does not protrude ventrally. Many small oil-like droplets, bluish in color, are distributed through the body, and give this cercaria a distinctive appearance. Numerous small spines are on the anterior end of the worm, but they thin out posteriorly so that they do not reach beyond the acetabulum.

The length of the tail averages about one-half that of the body. The tail is inserted on the ventral side of the body a short distance from the posterior end. When it is contracted there is a dark area in its center with occasional droplets and groups of cells scattered through it. The tail has no fin-fold and no caudal excretory tubule. Few nuclei are present in the tail. The ratio of the body length to the tail varies with contraction. All width measurements were taken through the region of the acetabulum. With faint pressure the contracted body is 0.129 mm. by 0.168 mm. and when extended it is 0.392 mm. by 73 μ . Measurements

*Since the publication of the abstract of this monograph in 1935, the writer has found that *Cercaria tetradena* E. L. Miller 1935 is a homonym of *Cercaria tetradena* Faust 1924. References to this species elsewhere in the present paper have been changed to *C. pachycystata*, as the writer is here proposing the name *C. pachycystata* for *C. tetradena* E. L. Miller 1935.

taken to show the ratio of the tail and body length showed the quiescent body to be 0.336 mm. by 0.157 mm. when pressure was used. At the same time the tail is 0.169 mm. by 30 μ . These measurements show the tail length to be about half that of the body. The greatest length noted is that of 0.47 mm. for the body and 0.28 mm. for the tail when they were both fully extended.

The stylet has thick walls which become thinner in its pointed anterior cone; however, it lacks the pronounced swelling or ridge which is so common to those forms which we speak of as Cercariae Armatae. Its length averages 16 μ and its distal two-thirds consists of a hard, thickened outer covering, which comes to a point gradually (Fig. 76). It is in the dorsal lip of the oral sucker.

The oral sucker is located almost at the tip of the body. Prominent circular muscle fibers line the cavity of the sucker. The two suckers are equal in size, averages showing the diameter of the oral sucker to be 59 μ , that of the acetabulum to be 52 μ . Under medium pressure the acetabulum is about 0.13 mm. posterior to the oral sucker and 0.153 mm. anterior to the posterior end of the body. A long prepharynx, 30 μ long, a comparatively large pharynx, 23 μ by 34 μ , and a short esophagus are present. Near the acetabulum the esophagus divides into two narrow non-functional crura which extend posteriad almost to the end of the body and slightly posterior to the excretory sphincter (Fig. 74). Only by persistent pressure, staining, and use of oil immersion, can one see these in the living form.

Two large ducts extend posteriad from the anterior dorsal margin of the oral sucker and connect with a group of four large glands on each side of, and anterior to, the acetabulum. Each duct consists of three large strands clearly separated for some distance at their anterior end. Near the edge of the oral sucker the three strands unite or at least become twisted about each other, but finally widen again before they connect with the salivary glands. A group of closely-packed, very small cells or glands are also located along the ventro-posterior edge of the oral sucker.

The excretory bladder is unusually complicated. Just anterior to the union of the ventrally attached tail is a large sphincter possessing very thick walls and hair-like structures on its inner postero-lateral walls. It empties into a narrow canal which extends to the posterior end of the body above the tail base and opens to the exterior at the end of the body between it and the tail. Anterior to this sphincter extends a thick-walled canal, the posterior portion of which periodically expands into a bladder during the functioning of the expulsion canal. This canal is irregular in its course during contraction. Just posterior to the aceta-

bulum it expands into another transverse vesicle which is about as wide as the acetabulum (Fig. 77). The lateral portions of this vesicle pulsate, forcing liquid down the lumen into the posterior bulb of the bladder.

Each lateral bulb connects laterally with a main collecting tubule which, at its lateral extremity, divides into an anterior and a posterior secondary collecting tubule. Each secondary tubule has three branches, the anterior branch of the posterior tubule being directed forward while the posterior branch of the anterior tubule is extended posteriad (Fig. 77). Each branch, at least in the posterior half of the body, divides into five capillaries. However, the penetration glands and other material in the anterior half of the body make it difficult to determine the number of capillaries and flame cells there.

A single genital mass lies immediately posterior to the acetabulum and is composed of minute cells which stain pink in neutral red.

The mature sporocysts have walls containing much orange or brownish pigment, and they fill all available space in the liver of the snail (Fig. 75). They are elongate sacs with blunt ends and thin walls, and contain many cercariae in various stages of development. The sacs are not filiform in the sense of having a length at all comparable to that of the sporocysts of *C. mesotyphla*.

Various features of the excretory system, the salivary glands, genital mass, and digestive system of this species differ sufficiently from all described species to verify my statement that the above form is a new species. While *C. acanthocoela*, *C. tricystica*, *C. cystonchnoides*, and *C. steganocoela* bear certain features in common with *C. pachycystata* that suggest possible relationship, nevertheless *C. pachycystata* differs materially from those forms. Another form, *C. pseudarmata* Brown 1926, differs regarding its excretory bladder. *C. leptosoma* Brown 1926 has five penetration glands on each side. *C. pandora* Faust 1921 has four penetration glands on each side also, but its rudimentary intestinal ceca differ radically from those of *C. pachycystata*. (See footnote on page 72.)

Cercaria tridena nov. sp.

(Figs. 78-80)

Snails collected at Baton Rouge, Louisiana, in April, 1932, and later identified as *Helisoma lantum*, were found infected with a small xiphidiocercaria, whose structure has points in common with that of *C. steganocoela*, *C. tricystica*, and *C. acanthocoela*. However, its digestive system and salivary glands differ noticeably from those of the above cercariae.

The worm does not encyst after emerging from the snail but soon settles to the bottom of the container where it crawls about for approx-

imately twenty-four to thirty-six hours before dying. It is not a strong swimmer, but moves rather aimlessly through the water with its body contracted, and bent ventrally, while its tail lashes vigorously. The tail is short as compared to the body when the worm is at rest, and it contains lateral folds when contracted.

When the oval body is contracted the region just anterior to the acetabulum is much wider than the region posterior to this organ. Therefore, all width measurements were made in this region. The anterior end is often extended into a snout-like process when it is elongated, and at that time the length of the oral sucker is greater than its width.

When at rest the body is 0.196 mm. by 0.106 mm., but may vary from 0.168 mm. by 0.129 mm. to 0.392 mm. by 56 μ . The tail varies in size also. When almost completely contracted it is only 33 μ long and 73 μ wide at its base, but it varies from 0.112 by 0.14 mm. to 0.252 mm. by 26 μ . Thus the tail is slightly shorter than the body (Fig. 79).

Tiny spines, much smaller than those of *C. acanthochoela*, are in the anterior region, particularly lateral to the oral sucker. They thin out posteriorly and become much smaller, so that in the region of the acetabulum they are contained entirely within the cuticula. From a point a short distance back of the acetabulum to the posterior end of the body, no spines can be seen.

The caudal pockets are very much reduced so that the lumen is almost absent. Setae could not be found on the walls of these pockets.

When contracted, the tail is very short, being only a fraction of the length of the body. It is attached to the ventral side of the body, and a short distance in front of the posterior end of the body (Fig. 78). A narrow lumen extends through the tail, and ends distally in an enlargement a short distance in front of the distal end of the tail. This lumen possesses irregular swellings throughout its course. When the body is quiet or crawling along by means of its suckers, the tail beats much of the time.

The oral sucker is only slightly larger than the acetabulum. When slightly contracted it is 44 μ long and 93 μ in width while the acetabulum is 52 μ long and 56 μ wide. When at rest the oral sucker is 61 μ long and 72 μ wide. At the same time the acetabulum is 59 μ long and 59 μ wide. Thus the oral sucker is slightly the larger. It bears a comparatively small stylet which has the thickened ridge, or shoulder, at the base of its narrowed anterior third. However, this ridge is less pronounced than in the majority of these forms. Its thickened walls extend backwards on either side of the stylet, and end in the region of the murky base which is a small area definitely differentiated from the rest of the stylet by the absence of a thickened wall or hollow center. The stylet is

20 μ in length and 4 μ in width at its base. The acetabulum is about midway between the oral sucker and the posterior end of the body. It is 49 μ posterior to the oral sucker when the body is contracted and 0.104 mm. when extended. It is also 52 μ in front of the posterior end when contracted, and 0.117 mm. while the worm is extended.

Posterior to the oral sucker is a very short prepharynx which is evident only when the worm is extended (Fig. 78), a comparatively large pharynx measuring 23 μ in both length and width, an esophagus reaching about half-way to the acetabulum, and intestinal crura which lie close to the lateral margins of the acetabulum when the worm is contracted, but describe a broad arc when it is extended. They, as well as the esophagus, are very slender, and in various regions consist of solid masses of tissue. However, the posterior regions are, in all cases, hollow. These crura extend 52 μ posterior to the acetabulum, or for about half the distance between it and the posterior end of the body.

Ducts from the penetration glands pass forward to a point on either side of the anterior tip of the stylet and open to the exterior. Under pressure a thick liquid can be seen slowly exuding from these openings. Posteriorly the ducts possess many enlargements, apparently due to their twisting, and connect to the glands in the region anterior and lateral to the acetabulum. Only three large glands could be seen on either side of the acetabulum, with occasional lobes occurring in one or more of them.

Dark granular masses, of a murky gray color, extend along either side of the body from the posterior end to a point a little behind the oral sucker. These may be cystogenous glands. Other globular bodies are scattered over the body, most of them being anterior to the acetabulum. They are lighter in color than the penetration glands, and have definite clear walls. Genital cell masses lie immediately anterior and posterior to the acetabulum and consist of elongated masses of small spherical cells (Fig. 78).

The excretory bladder extends forward from the excretory pore almost to the acetabulum before dividing into lateral arms to form a broad Y. As it contracts this bladder may constrict at the anterior end of its stem; the fluids of the arms flow together, and there then appear to be two separate vesicles, a caudal and an anterior one. When the body is contracted these arms form an irregular transverse vesicle. However, I have never noticed the separated lateral bladders appearing as in *C. acanthocoela* and *C. tricystica*. A greatly twisted main collecting tubule divides lateral to the acetabulum and slightly behind it to form a posterior and an anterior secondary collecting tubule. Just anterior to the acetabulum the anterior tubule divides into two smaller branches, but due to the glandular nature of the body it is impossible to trace the tubules of the excretory system further.

The liver tissue of *Helisoma lantum* was literally replaced by an immense tangled mass of short, oblong sporocysts, with compact, smooth outlines (Fig. 80) rather than irregular ones as is characteristic of the sporocysts of many other Cercariae Armatae. Some older sporocysts are light gray in color but most of them are a brilliant orange. Many of these sporocysts contain encysted forms which will be described later. The sacs average 0.756 mm. in length and 0.14 mm. in width.

In one of these sporocysts a large overgrown cercaria was found much like the one found in the sporocyst of *C. acanthochoela*. This individual was tailless and had lost its stylet, but was filled with a great mass of material resembling cystogenous material which obscured most of the structures in the body. Its bladder was greatly swollen with excretory granules as is the bladder of the metacercariae, the digestive system was plainly visible with only slight pressure, and the worm moved slowly as does a metacercaria after being liberated from its cyst. This worm is 0.246 mm. long, when mediumly contracted, and 0.151 mm. wide. The oral sucker is 62 μ wide and 59 μ long while the acetabulum is only 49 μ long and 53 μ wide. The acetabulum is about 65 μ posterior to the oral sucker and 72 μ anterior to the posterior end. These measurements may mean that this individual indicates a dual infection but I do not believe the differences noted here warrant such a conclusion.

As previously mentioned, this form encysts readily within the sporocyst and liver tissue of the host. However, many never encyst, as shown by the fact that the encysted forms are present in relatively small numbers, in comparison with the immense number of cercariae that emerge.

The cyst wall is a clear, transparent, tough substance; and when the wall is broken, the worm begins to move about. Eight ducts open separately, dorsal to the cavity of the oral sucker. A group of tiny, elongated, tube-like structures was noted here in the cercaria before it encysted, but because of their minute size these structures could not be carefully studied. Distinct spines are on the anterior portion of the body, but become smaller posteriorly until, in the posterior region of the body, they cannot be seen. The excretory bladder is now a large sac-like structure filled with granules and the crura reach farther posteriorly than they did in the unencysted form. The lateral bodies, possibly vitelline follicles, present in the cercaria are now more pronounced, and many large cells with prominent nuclei are scattered through the body.

Another form, the cercaria of *Lissorthis fairporti* described by Magath (1917), resembles *C. tridena* superficially but cannot be mistaken for it because of the difference in the salivary glands and excretory bladder. The structure of its stylet is also different. *C. crenata* Faust 1917 differs from *C. tridena* in the number and arrangement of its

salivary glands, in the possession of two very unequal suckers, shorter digestive ceca, and various other characters of less importance. *C. leptosoma* Brown 1926 has five mucin glands on each side of its body.

IX. FURCOCERCOUS CERCARIAE

Discussion of the Group

The first furcocercous cercaria described was that one given the name *Vibrio malleus* by Müller in 1773. *Cercaria vivax* Sonsino 1892, one of the first to be described outside of Europe, was also one of the very earliest to receive a detailed description (Looss, 1896). Lühe (1909) briefly described the furcocercous larvae in Germany and neighboring countries but grouped two of these individuals with his Lophocercariae because they were monostomes. He described the furcocercous cercariae as follows:

Distome Cercarien mit langem, an seinem freien Ende gegabelten Schwanze, in welchen der schlanke Körper nicht zurückgezogen werden kann. Entwicklung meist in sehr langgestreckten Sporocysten, welche (ob bei allen Arten?) selbständig beweglich sind, nur bei einer Art angeblich in Redien.

Lühe divided these forms on the basis of presence or absence of eye-spots. Lebour (1911) divided the British marine forms into two primary groups on the basis of development in sporocysts or rediae. Cort described the first form reported from North America, *Cercaria douthitti* Cort 1914.

Because of Leiper's work (1915) the schistosome cercariae were separated from other furcocercous forms by their lack of a pharynx, of pigmented eye-spots, and of a cuticular keel on the furcae, which are less than one-half as long as the tail stem. Cort (1917) divided several of these forms into three groups on the basis of presence or absence of pharynx and eye-spots but his scheme is too limited to allow the inclusion of all forms now known. Sewell (1922) gave the first complete survey of all furcocercous cercariae but he separated out all monostome forms since he considered the presence or absence of an acetabulum to be of prime importance here in a system of classification. However, McCoy (1928, 1929a) found that furcocercous monostomes may develop into adult distomes.

Faust (1924) divided these forms according to the excretory system's flame cell pattern because he believed it was a natural basis for such grouping. His belief that it is a common system carried over from the cercaria to the adult would seem to be refuted by Miller's statement (1926a) that flame cells of the cercaria might have potentialities for more rapid division such that the pattern of the adult worm could not be predicted. This has been illustrated in other forms, for instance, in the larval and adult *Schistosoma japonicum*.

Miller (1926a) in his comprehensive studies on furcocercous larvae divided all known forms into two main groups, the Apharyngeal Cercariae and the Pharyngeal Cercariae, which probably constitute natural groups. Each of these were subdivided according to whether the individuals were brevifurcate or longifurcate and distomes or monostomes.

The species included in my studies will be listed in their proper position in Miller's scheme of classification.

APHARYNGEAL BREVIFURCATE DISTOME CERCARIAE

Definition

All furcocercous cercariae with no pharynx, with furcae which are usually less than one-half the tail-stem length and frequently sharply delimited from the tail-stem and which have both a ventral and oral sucker were placed in this group by Miller (1926a). He defined the brevifurcate larvae as follows:

Furcae usually less than one-half the tail-stem length; frequently sharply delimited from the tail-stem. Tail-stem diameter less than that of body; usually attached somewhat ventrally, sometimes decidedly so, such that a dorso-ventral mount is rare. Furcal fin-folds sometimes present. Body frequently very hyaline. Eye-spots may be present. Anterior organ a very highly modified oral sucker, with anterior thin-walled and posterior muscular portions; head gland usually present. Ventral sucker usually much smaller in diameter than anterior organ; very protrusible and often held protruded. Penetration glands very large; frequently divided into anterior coarsely granular and posterior finely granular cells. Penetration gland duct openings frequently capped by hollow piercing spines. Excretory openings at tips of furcae. Never more than one pair of flame cells in proximal part of tail-stem. Tail-stem wall usually provided with powerful longitudinal muscles. Caudal glands not conspicuous; when present, are not arranged in pairs along caudal excretory tube. Tail-stem and furcae usually spined; no sensory hairs. Furcae almost cylindrical in some larvae. Alimentary canal opens more or less ventrally as a capillary tube; ceca absent or at most very short.

Miller (1926a) divided the apharyngeal brevifurcate distomes into eight subgroups and designated them as Subgroup A, B, C, D, E, F, G, and H. He listed twenty-six such forms and I have found descriptions of additional ones, namely, *Cercaria syncytadena* Faust 1926 and *Cercaria anomala* Rao 1929.

Cercaria wardi Miller 1923

(Figs. 81-87)

During my studies of infected *Helisoma trivolvis* collected in an Ox-bow north of St. Joseph in June, 1932, a unique furcocercous larva was discovered which was described and named by Miller in 1923, and later discussed in his monograph on the furcocercous cercariae. Additional information about this form may be of value.

The body is very thick so that usually it must be viewed from the side under the cover-slip, only rarely a dorsal or ventral view being obtainable. The body is humped above the tail insertion (Fig. 81) as is also that of *Cercaria gigas* Faust 1918. Spherical eye-spots are the most prominent features of the worm. Large prominent backward-pointing spines cover the anterior end of the worm, forming a thick crown over the front half of the swollen anterior portion of the oral sucker (Fig. 86) and reaching posteriorly for a distance of about 46 μ .

The body is broadly subquadrate at its posterior end, and narrowed anterior to the acetabulum. When the body is under medium pressure my measurements are as follows: Body length, 0.35 mm. and greatest width at the posterior end, 0.156 mm.; tail stem, 0.689 mm. long, 65 μ wide at base, 85 μ wide at its middle; furcae, 0.247 mm. long and 52 μ wide near base. These measurements slightly exceed Miller's.

The oral sucker is pyriform in shape (Fig. 86) with the posterior end smaller. It is about 0.13 mm. long by 68 μ in width at its anterior end and 39 μ at its posterior end. The acetabulum protrudes prominently and is easily everted by slight pressure. Its inside is thickly covered with small spines and the entire body contains tiny spines, those nearer the posterior end being embedded in the cuticula. The acetabulum is much smaller than the oral sucker, being only 50 μ in diameter. It is situated at the end of about the anterior two-thirds of the body. The esophagus extends ventrad and soon divides into the two intestinal crura which extend to the acetabulum (Fig. 81).

The circular eye-spots consist of spherical pigment granules which appear as a black mass in a cavity containing a transparent fluid. However, when these granules are scattered by pressure they are seen to be brownish in color. They are about 0.182 mm. posterior to the anterior end and 78 μ anterior to the acetabulum. Each measures 26 μ in transverse diameter and 20 μ in longitudinal diameter.

Many glands and other tissue fill the entire body of this cercaria. Penetration ducts, each consisting of several distinct parts, which open near the ventral border of the anterior margin of the oral sucker, emerge from the sucker ventrally and connect with a mass of five glands on each side of the body. They are clear in appearance and lie dorsal to the acetabulum in large part, immediately behind the intestinal crura. A large, irregular, granular mass lies just posterior to these glands and nearly fills the body space posterior to the acetabulum. This is the posterior mucin body formerly mentioned by Miller (1926a).

Just anterior to the posterior mucin gland, between it and the ventral sucker, is a germinal cell-mass. Surrounding the posterior mucin gland and particularly numerous dorsally, are many large gland-like cells, each with a large nucleus. These may be cystogenous in character. Authors

have used various designations for these bodies. The anterior region of the body is entirely obliterated after treatment with neutral red, because of these dark nuclei.

When quiescent the tail stem is twice as long as the body but it may be much longer than this when it is fully extended. The furcae, usually contracted, are a little shorter than the body. The stem is narrowed both distally and proximally and is attached to the ventro-posterior margin of the body. It is parallel with the longitudinal axis of the body. Muscle fibers are prominent and have been described by Miller (1923, 1926a). The furcae are slightly shorter than the body when quiescent under pressure but in this state they are always contracted so that the furcal fin-folds become fluted. Two dorso-ventral fin-folds extend along each furca throughout its length (Fig. 83) and possess numerous ray or rib-like structures, which are prominent at the end of the fin-fold (Fig. 82).

The study of the excretory system proved to be difficult since this system is obscured by the glands and other cells of the body. Two giant flame cells near the base of the tail stem constitute the most prominent feature of the tail. Measurements which include the basal or nucleated portion of the flame cells show it to be about $20\ \mu$ long while the flame itself is only $13\ \mu$ long. This vibratile portion becomes inactive when great pressure is applied and can then be studied in some detail. Even camera lucida drawings can be made during this stage (Fig. 87). The vibratile portion is clearly striated but there is no indication of there being separate cilia here. Four distinct dark bands, run longitudinally and three narrow, lighter striations separate them from each other. In motion this structure resembles the undulating movements of a vibratile membrane. The complete excretory system was determined (Fig. 84) and corresponds to the description given by Miller (1926a).

When the snail was dissected the liver tissue seemed to be a living mass of cercariae for the walls of the sporocysts are so thin, delicate, and transparent that they are very easily broken. I have never disentangled a complete sporocyst, but in a few cases have found parts of the sacs which contained maturing germ balls and cercariae (Fig. 85).

Miller placed this form in his Group E of apharyngeal brevifurcate distomes since all members of this group are unique in possessing a posterior mucin gland.

Cercaria pteractinota E. L. Miller 1935

(Figs. 88-93)

Specimens of *Physa gyrina* collected at Twin Lakes near Paris, Illinois, and at Baton Rouge, Louisiana, were found to be infected with a

large brevifurcate form; those of *Helisoma trivolvis*, collected at Camp Creek at Seymour, and at the Oxbow Lakes at Urbana and St. Joseph, were also infected with this cercaria. It hangs in the water with its head downward when not disturbed but can move rapidly forward by its tail movements. It has exceptional ability to extend itself, and the anterior end is sometimes drawn out to form a narrow neck, extending by an out-pushing process which is probably due to the ability of the anterior organ itself to extend. Under the cover-slip the long tail is a hindrance to locomotion by the suckers. The worm can live in tap water for a long time, having been observed in the water for a period of thirty-one hours, at the end of which several of the worms were still living.

The body is quite rectangular in shape, being wider at the posterior end than at the anterior, when even slightly contracted. It is long and comparatively narrow and may appear quite ribbon-like when completely extended (Fig. 92). The tail is nearly three times as long as the body when it is extended. The quiescent body is 0.27 mm. in length, and 0.363 mm. long when extended. The widest portion, just posterior to the eye-spots, is 0.104 mm. The tail stem is 0.532 mm. long and 56 μ wide, and the furcae are 0.185 mm. long and 28 μ wide at their bases.

An immense number of tiny spines covers the body but those over the anterior half of the oral sucker, or anterior organ, are much more prominent (Fig. 91). They are arranged in longitudinal and transverse rows but spaced so as to make diagonal rows as well.

The eye-spots are prominent features of the cercaria and are elongated transversely. They lie midway between the oral sucker and the small acetabulum. When the worm is at rest they lie 96 μ posterior to the anterior end, and when it is extended, about 0.156 mm. posterior, and 0.195 mm. from the posterior end. The two conspicuous black spots are large in comparison with the size of the body, being in width about one-third that of the body in that region. An eye-spot is about 39 μ in width and 12 μ in length. A small compact mass of pigment, which resembles the eye-spot of a planarian worm, lies in the center of each pigment mass. Two other prominent pigment masses are present, one on either side of the oral sucker near its posterior end. Other scattered brownish masses of pigment are distributed over the body, several of them being constant in position (Fig. 91).

When the worm is at rest, the distance from the base of the tail to its bifurcation may be nearly three times as great as the length of the body. A sharp division separates the tail stem from the furcae. These furcae bear fin-folds on both sides of the distal one-half to two-thirds of their length and each fold has numerous rays or ribs throughout its length (Fig. 90). A broad central lumen runs longitudinally through

the tail stem, extending also into the furcae, and is evident when little pressure is applied. The tail is inserted on the ventral side of the body and makes a noticeable angle with the long axis of the body because of this insertion. A membrane passes ventrally over the base of the tail, making a cup into which the tail fits. Longitudinal muscles run in a diagonal direction in the tail, both outer and deeper fibers being present. Two giant flame cells, very prominent, are in the basal portion of the tail stem.

The digestive system leads posteriad from the elongated oral sucker, no pharynx or prepharynx being present. The oral sucker is much larger than the ventral, measuring about $91\ \mu$ in length by $59\ \mu$ in width, while the diameter of the acetabulum is only $29\ \mu$. The oral sucker is sub-terminal and fills the whole anterior tip of the body (Fig. 93). The small acetabulum is on the tip of a prominent knob and is circular, easily everted with pressure, has its inner surface covered with spines, and has a three-cornered opening. It lies $65\ \mu$ posterior to the oral sucker and about $0.124\ \text{mm.}$ anterior to the posterior end of the body. The esophagus runs posteriad and ventrad but it could be traced in the living specimen for only a very short distance. It turns downward and runs ventrad to the two eye-spots, along the body wall.

Large ducts, tripartite in their anterior regions, pass posteriad over the oral sucker and extend ventral to the eye-spots, passing to a point slightly posterior to the acetabulum, where they communicate with the penetration glands. These glands fill a large portion of the body posterior to the acetabulum. They occupy the dorsal regions of the body, are granular, and distinguish themselves easily from the large lighter glandular bodies which fill most of the remaining space posterior to the eye-spots. However, under pressure these glands seem to fill the ventral regions posterior to the acetabulum.

Two or three ventral compact masses of dark cells lie posterior to the acetabulum in the ventral region of the body. Two medium-sized compact bodies also lie just above the acetabulum. These bodies probably represent the germinal masses of the cercaria. The anterior regions of the body are filled with tiny nuclei.

The excretory bladder is almost forked by an anterior mesial projection which incompletely divides it. The lateral main collecting tubules describe an elongated loop (Fig. 88) and then divide into anterior and posterior collecting tubules. A large median excretory tubule runs through the tail to its fork where the tubule also divides, one fork running into each of the furcae and extending to the very tip where it ends in a slight enlargement. Two giant flame cells are situated a short distance distal to the base of the tail, each connecting proximally with a tiny collecting tubule which runs through the base of the tail into the body.

The sporocyst of this form is a thin-walled, filiform sac. The walls of these sacs are so delicate that the separation of a single complete specimen from the tangled mass of the liver tissue of the host is extremely difficult. The walls are thin and easily broken so that it is not unusual to see the tail or body of an immature cercaria protruding through a break in the wall. The dark pigment granules sometimes give the walls a light grayish appearance but in most cases they are quite transparent. The sporocyst is so narrow that rarely more than a single cercaria lies lengthwise in the central cavity (Fig. 89). Several large cercariae and germ balls were found in the sacs in various stages of development. The powerful tail is usually coiled lengthwise in the sporocyst. The immature forms lack pigment except for the two large eye-spots posterior to the oral sucker.

Several of these forms were found encysted in the liver of the snails but in all cases the worm inside was dead. Undoubtedly this is not a normal procedure. Attempts to recover encysted forms of *C. pteractinota* from crayfish were not successful.

C. pteractinota belongs to Miller's group of apharyngeal brevifurcate distome cercariae. The relatively few penetration glands and the absence of eye-spots in all members of Group A prevents me from placing this form there, while Group B also is characterized by only a few of these glands, only five pairs. The small size and presence of a few penetration glands in members of Group C also separate them from this form. *C. pteractinota* lacks the posterior mucin gland of Group E, described above for *C. wardi*. The five distinct penetration glands and the posterior cell mass in members of Group G prevent me from listing *C. pteractinota* with these forms. *C. elephantis* Cort 1917 has a body and tail covered with spines and *C. echinocauda* O'Roke 1917 develops in rediae. *Cercaria gigas* Faust 1918 of Group H, has a much shorter tail stem, has flutings in the furcae instead of rays, has two distinct types of glands, lacks the pigment masses described for *C. pteractinota*, and has only a single germinal mass of cells. *C. pteractinota* must therefore be placed in Group F since it lacks the so-called posterior mucin gland and the head gland found in Group E. The possession of numerous pigment masses, of numerous penetration glands, of two germ masses instead of one, and of elongated sporocysts, instead of small oval ones, separates this new species from *Cercaria indica* xxxvi Sewell 1922, the only representative of Group F. More recent apharyngeal brevifurcate distomes such as *C. anomala* Rao 1929 and *C. syncytadena* Faust 1926 bear only a superficial resemblance to *C. pteractinota*.

Cercaria gigas Faust 1918

(Fig. 94)

This form was found only once in *Physa gyrina* collected in the Sangamon River near Mahomet, Illinois, on May 29, 1932. Formerly Faust (1918) reported this in *Helisoma trivolvis* and *Physa gyrina* from DeKalb, Mt. Morris, and Urbana, Illinois. Few additions to Faust's original description can be made since the snail host soon died after it was brought into the laboratory. The large, characteristic, pigmented eye-spots lying at a point about one-third of the body length from the anterior end (Fig. 94), the absence of a pharynx, the presence of a small, spined, protruding acetabulum, the long tail stem with very short furcae, the snout-like, elongate oral sucker which is also covered with spines, the gland cells posterior to the acetabulum, and the large number of penetration glands all help to identify this form as the sole member of Group H of Miller's apharyngeal brevifurcate distome furcocercous cercariae.

PHARYNGEAL LONGIFURCATE MONOSTOME CERCARIAE

Definition

About one-half of the furcocercous forms possess pharyngeal sphincters. Miller (1926a) separated the longifurcate from the brevifurcate cercariae on the basis of the following description:

Furcae longer than one-half the tail-stem, sometimes exceeding it; usually not sharply delimited. Tail-stem diameter approximately equal to that of body when fully extended; attached terminally, dorso-ventral mount the usual one. Furcal fin-folds absent. Body usually crowded with small parenchyme cells. Eye-spots usually absent. Anterior organ less highly modified; cells which possibly represent a head gland present in but a few larvae. Ventral sucker frequently large, in some cases of greater diameter than anterior organ. Penetration glands small in proportion to body; no differentiation into anterior and posterior sets. Usually coarsely granular, and acidophilic in sections. Solid piercing spines more frequent than hollow ones. Excretory openings typically mid-furcal. Usually two pairs of tail-stem flame cells, seldom confined to a strictly proximal location. Tail-stem wall frequently annulated; tail less powerful and more transparent. Conspicuous, more or less regularly paired caudal glands in a number of species. Tail-stem usually devoid of spines; furcae may be spined; sensory hairs on the tail-stems of several larvae. Furcae never cylindrical, usually much flattened. Alimentary canal usually opens terminally or subterminally; esophagus a fair-sized tube; ceca usually well-developed, frequently reaching almost to posterior end of body.

Miller also divided the pharyngeal longifurcate monostomes into three groups, the Vivax Group, the Tetis Group, and the Rhabdocaeca Group, and listed eight species as belonging to the three groups. Since then other forms have been described, namely, *Cercaria dorsocauda* Tubangui 1928, *Cercaria bessiae* Cort & Brooks 1928, and *Cercaria physae* Cort & Brooks 1928. The character of its excretory system indicates that *C. dorsocauda* undoubtedly belongs in the Vivax Group. *C.*

bessiae and *C. physae* belong to the Rhabdocaeca Group because they contain six posterior penetration glands instead of the numerous anterior ones characteristic of the Vivax and Tetis Groups. They also agree with other members of this group in possessing sensory hairs on the tail stem instead of spines, and in having an excretory system slightly different but still characteristic of the group.

The following forms have been placed in the Rhabdocaeca Group:

- | | |
|---|---|
| 1. <i>Cercaria rhabdocaeca</i> Faust 1919 | 4. <i>C. dorsocauda</i> Tubangui 1928 |
| 2. <i>C. hamata</i> Miller 1923 | 5. <i>C. bessiae</i> Cort and Brooks 1928 |
| 3. <i>C. multicellulata</i> Miller 1923 | 6. <i>C. physae</i> Cort and Brooks 1928 |

Study of *C. hamata* and *C. multicellulata*, collected in Illinois, and of *C. bessiae*, collected in Louisiana, has been made. Also descriptions of two new species of these pharyngeal longifurcate monostomes will be given here, one species from Illinois and the other from Louisiana.

Cercaria hamata Miller 1923

(Figs. 95-98)

This cercaria was found in *Helisoma trivolvis* collected in the Oxbow Lakes near Urbana and St. Joseph, and in *P. gyrina hildrethiana* in Lake Decatur near Decatur, Illinois. Miller (1926a) reported it from the Urbana area. The worm lies in the water with its anterior end bent ventrally, giving the hook-shaped appearance characteristic of so many of these forms. Annulations appear as the worm contracts slightly but they are confined largely to the anterior one-half or three-fourths of the body surface. In locomotion the tail functions so that the cercaria swims backwards by jerky spasmodic movements.

Normally the stem and furcae are of about the same length, the body being slightly shorter. The width of the tail stem is equal to that of the body unless the body is greatly contracted. Under pressure the body is 0.224 mm. long, the tail stem 0.252 mm. long, and the furcae 0.235 mm. long. The body is 45 μ wide at its greatest width, the tail stem is 39 μ and the furcae 28 μ at their bases. However, average measurements for slightly extended specimens are: body 0.151 mm. long, tail stem 0.213 mm. long, and furcae 0.209 mm. long. Spines are present on the anterior tip of the body but do not extend much posterior to the region of the oral sucker.

An elongate, relatively large, terminal oral sucker empties into a very narrow, undeveloped prepharynx and tiny bulbular pharynx (Fig. 95). The anterior organ is not pyriform as it is in *C. multicellulata*. The short rhabdocoele gut represents the development of the digestive system beyond the pharynx. The oral sucker is 29 μ long and 33 μ wide when contracted and 39 μ by 23 μ when extended. No acetabulum is present but a circular mass of cells which separates the anterior pair of penetra-

tion glands from the two posterior pairs may be the undeveloped sucker. The pharynx is 12 μ in width and 9 μ in length while the prepharynx is about 12 μ long.

A group of six large clear nucleated penetration glands lies in the posterior region of the body. The three glands of each side are arranged in linear fashion and taper into the narrow ducts leading from each. When the body is contracted these glands become globular or even broadly rectangular with the transverse dimension greater than the longitudinal, but with body extension they become duct-like in appearance. A single clear vesicular nucleus is in the center of each gland. Each duct divides just in front of the anterior pair of glands, one duct passing to the anterior gland and the other dividing to connect with the two posterior glands (Fig. 95). Anteriorly the ducts continue to the oral sucker and pass through it, where they become greatly swollen and the two parts of the duct become distinctly separated. They open at the anterior end where they are associated with large, definitely pointed spines. For some distance posterior to the pharynx these ducts are indistinct in the network of glandular bodies and tissue there.

A mass of deeply-staining cells in neutral red, the germinal mass, is posterior to the penetration glands and anterior to the excretory bladder. Many small round nuclei are scattered over the body.

A small, constricted excretory bladder lies at the posterior end of the body; it is constricted near its middle so as to give the appearance of a double bladder. Extending posteriorly from this bladder is a single median tubule which runs along the longitudinal axis of the tail-stem and divides near the fork (Fig. 98). A small island of Cort is present. Each branch of this tubule extends through one-half the distance of the furca before ending in a solid rod of cells near its lateral border. I have never seen external openings for these tubules as described by Miller, but have seen a terminal solid mass there in some specimens. Anteriorly each of the main collecting tubules of the body divides into an anterior and posterior branch. The anterior ends in four capillaries, each with its flame cell, and the branches of the posterior end in six flame cells, four of which are in the body and two in the tail-stem (Fig. 95).

The tail-stem is attached terminally as in *C. multicellulata*. It bears numerous sensory hairs which number about fifteen or sixteen on each side (Fig. 96). Numerous caudal glands are present which become greatly flattened and distended under pressure. A wide, glandular lumen extends through this stem and divides to extend into the tips of the furcae. Many nuclei lie in the tail, both in the stem and furcae, and, from a surface view, are evenly distributed. Loose muscle bands extend throughout the length of the tail-stem and terminate in the furcae.

The furcae are very long, each as long as the tail-stem and longer

than the body when at rest (Fig. 98). No fin-fold is present. From four to six striations extend longitudinally through the furcae, the six distal striations decreasing in number near the basal region, until, at the proximal end, there are only four such lines. These striations do not occur in the tail-stem. They are easily seen in the living, unstained specimen after strong pressure has been used but cannot be seen in stained forms since the furcal nuclei obscure them. Two rod-like structures (Fig. 97) arise at the posterior border of the furcal union and pass through about half the length of the furcae before ending near the outer margin of the furcae in a tiny mass of solid tissue. Occasionally one of these passes to the inner margin instead of to the outer. Earlier in this study they were believed to be the furcal excretory tubules but their apparent permanence under pressure makes me believe they represent different structures.

Great masses of slender, thread-like parthenitae fill all available space within the outer membranes of the liver of the host. The sporocysts are attenuate structures and resemble a mass of slowly oscillating algae because of their power of independent movement. They can contract and extend and when contracted, swollen, bulb-like areas are present. They vary in color from orange to brown and contain developing larvae and loose tissue in their centers. Each is swollen at one end, and is unusually active in its movements, its tip contracting much as the anterior end of an adult trematode. These bodies vary greatly in size. Narrow portions of the sac are only $28\ \mu$ in width while the swollen active ends are $84\ \mu$ in width. The sporocyst averages about $56\ \mu$ in width and varies in length from 2.0 mm. to 9.73 mm. Many of the smaller ones are 3.5 mm. long. The birth pore mentioned by Miller was not observed.

C. hamata differs from *C. multicellulata* and *C. physae* in that it lacks the pigmented eye-spots which the latter forms have. *C. bessiae* has unpigmented eye-spots and also has no caudal bodies. No pharynx has been described for *C. multicellulata*. The possession of a well-developed digestive system, of a different excretory system, and of a radically different body outline differentiates *C. dorsocauda* from this form. *C. rhabdocaeca* lacks glands and sensory hairs on the tail-stem. Its bladder is very different from that of *C. hamata* and it has no island of Cort. The oval anterior organ, the tail-stem of uniform width, the limited body spines, the caudal bodies and sensory hairs, the island of Cort and body proportions all enable one to recognize *C. hamata*.

Cercaria multicellulata Miller 1923

(Fig. 99)

The *Physa gyrina hildrethiana* found to be infected with this cercaria

were collected at Seymour, Illinois, in Camp Creek, in April, 1932. Miller (1926a) reported it from Urbana, Illinois.

The cercaria hangs in the water with its furcae above and open. It may dash suddenly through the water with the furcae working so as to draw the body after them. The body is shorter than the tail-stem and the furcae, but not noticeably so. The tail-stem is slightly longer than the furcae when they are quiescent. Small, thickly-set spines cover the anterior end of the oral sucker but thin out rapidly posteriorly, reaching only to a point about midway between the anterior organ and the eye-spots. These small, pigmented eye-spots are about midway between the oral sucker and the posterior end of the body.

The tail is attached terminally and its stem and furcae are filled with many large nuclei. Caudal bodies or glands are also present in the stem (Fig. 99) and are much more easily seen in some individuals than in others, depending on the age of each particular cercaria. Small, narrow fin-folds extend along the sides of the furcae.

No trace of a pharynx or alimentary canal is evident. The large oral sucker is terminal and easily evertible, this action being repeated continually under observation.

The yellow, thread-like sporocysts were so tangled that individuals were not completely separated for study. Many parts of sporocysts were studied and found to be filled with germ balls and undeveloped cercariae in different stages of development.

The pyriform oral sucker, the extent of the spination, the two pigmented eye-spots, the uniform tail-stem, the thin furcal edges, the sensory hairs of the stem, the absence of a digestive canal or pharynx, the paired caudal bodies, the excretory system, and the mass of cells which separates the anterior pair of penetration glands from the two posterior pairs, which may be the rudimentary ventral sucker, all help to distinguish this form.

The absence of a pharynx separates this from other pharyngeal longifurcate monostomes. It also differs from all except *C. bessiae* and *C. physae* in the possession of eye-spots. Those of *C. bessiae* are not pigmented. *C. physae* has only five pairs of caudal bodies while *C. multi-cellulata* usually has about eight pairs.

Cercaria bessiae Cort & Brooks 1928

(Figs. 100-101)

The material which furnished the basis for the present study of this species was found at Baton Rouge, Louisiana, in April, 1932, in *Helisoma lantum*. Cort and Brooks have reported it from Douglas Lake, Michigan, from *Helisoma trivolvis*.

The cercaria hangs fairly motionless, head downward in the water for rather long intervals; then the furcae suddenly flip rapidly and the worm shoots upward in the water, the tail foremost. When at rest the body is characteristically bent downward like a hook, as mentioned by Cort and Brooks.

The body is elongated, with a round, narrow anterior end and sub-quadrate posterior end. Under slight pressure and when quiescent the body is no wider than the tail-stem. When at rest the body is usually slightly shorter than the tail-stem and furcae, while they are of about the same length. When neither extending nor contracting under the cover-slip the body measures 0.168 mm. by $40\ \mu$, the tail-stem from 0.196 mm. to 0.241 mm. by $45\ \mu$, and the furcae 0.185 mm. by $22\ \mu$ at their bases. When the body has the same width as that of the tail stem due to extension it is about 0.224 mm. long.

Under high power a group of thickly-set spines can be distinguished at the anterior tip of the body but they thin out rapidly posteriorly, not extending beyond the anterior third of the body. Annulations, due to the contraction of circular muscles, are present on the anterior portion of the body. Two unpigmented eye-spots are located just anterior to the first pair of penetration glands (Fig. 100).

The tail-stem has a nuclear core which extends through its longitudinal axis and also into the furcae. This stem is inserted in the body terminally, it lacks caudal bodies but has from six to ten sensory hairs on each side. Surface nuclei are also present in the stem, and a single focus indicates that they are distributed regularly along either side of the stem, and through most of the furcae. Fine striations extend from the bases to the tips of the furcae of the tail; six rows or lines commonly are present.

The oval anterior organ is $33\ \mu$ by $20\ \mu$; it is followed by a short prepharynx, a small bulbular pharynx about $10\ \mu$ long, and a short rhabdocoele intestine. A group of minute cells which separates the two anterior penetration glands from the four posterior, is interpreted as the rudimentary acetabulum.

The genital primordium at the posterior end of the body just anterior to the excretory bladder consists of a large mass of compact, deeply-staining cells in neutral red.

The two penetration ducts open at the anterior end in close association with the numerous spines mentioned previously. Each duct is bipartite, is swollen in its course through the anterior organ, and extends posteriad to connect with the penetration glands. Near the posterior edge of the oral sucker these ducts decrease noticeably in size, but increase in their posterior regions again where they lie close together, near the median plane of the body, when the worm is not subjected to pressure. When

pressure is applied they are pressed some distance apart and define a wavy regular course. There are three penetration glands on each side in the posterior region of the body. One-half of each mucin duct connects with two posterior glands, running under the anterior gland, while the other half of the duct connects with the anterior gland. The large, clear nuclei are numerous in the body of the living worm.

The bladder has a lateral constriction so that it is partially divided into a small posterior and a large anterior part. Lateral collecting tubules divide to form a posterior and an anterior secondary tubule near the anterior penetration gland and branch in the manner described by Cort and Brooks (1928). I have not succeeded in determining the connections of the third pair of flame cells because of the parenchyma which obscures this region.

Cort and Brooks did not describe the sporocyst of this form. The liver tissue of *Helisoma lantum* is filled with very long, narrow parthenitae (Fig. 101) which occupy all available space. Few developing forms are in the central cavities but granular material is profuse. Brown and orange pigments give these sacs their characteristic color, which is of a deeper brown in the swollen parts of the sac. The ends are swollen and the outline of the entire sporocyst is irregular, due to swollen areas alternating with constricted neck-like regions. It is very difficult to remove a single complete sporocyst from the tangled mass in the liver of the host. The narrowest width observed in the sporocyst was 28 μ , the average width was about 56 μ , and the width of the swollen end was 0.112 mm. The largest forms are 5.6 mm. in length.

This is the only species of the pharyngeal longifurcate monostome forms which has unpigmented eye-spots. Other differences between these species which prove to be of value in separating *C. bessiae* are: The possession of caudal bodies by *C. hamata*, *C. multicellulata*, and *C. physae*; the spines over all the body and tail of *C. dorsocauda*; the absence of a pharynx in *C. multicellulata*; and the different excretory system and large island of Cort in *C. rhabdocaeca*.

Cercaria furcalineata nov. sp.

(Figs. 102-104)

Helisoma lantum collected at Baton Rouge, Louisiana, in April, 1932, were found to be infected with a pharyngeal longifurcate monostome cercaria different from all those of this group previously described.

It hangs in the water in a manner similar to that described for *C. bessiae*, and its anterior end is bent downward to form a characteristic hook (Fig. 103). Its method of locomotion is similar to that of *C. bessiae*.

The body is not greater in width than the tail-stem when at rest or extended. When at rest the body is about 0.168 mm. long by $39\ \mu$ through its greatest width, the tail stem is 0.28 mm. long and $39\ \mu$ wide, and the furcae are 0.252 mm. long and $22\ \mu$ wide at their bases. When contracted the body is 0.101 mm. long and $56\ \mu$ wide. Under strong pressure with the body only slightly contracted it is 0.111 mm. by $72\ \mu$, the tail stem is 0.247 mm. by $42\ \mu$, and the furcae are 0.247 mm. by $26\ \mu$. These measurements indicate that the body is shorter than the tail-stem or the furcae, and that the stem and furcae are of about the same length.

Spines are present at the anterior end but were not seen in the regions posterior to the oral sucker.

The tail is attached to the ventro-posterior tip of the body. The stem and furcae contain a great number of scattered nuclei which are most abundant in the lumen and along their sides. Five or six striations extend lengthwise through the furcae and resemble those which I have previously described for other pharyngeal longifurcate monostomes (Fig. 104). These broken lines have not been mentioned by other workers. I know of no function which they might perform except possibly that of giving rigidity to the furcae. They do not extend above the base of the furcae. A single, broken, linear-like rod extends outward from each side of the furcal union and ends near the outer wall of the furca. The terminations vary in different individuals and may be occasionally on the inner wall of the furcae (Fig. 103). Possibly these rods also give rigidity to the furcae. They differ from those described for *C. hamata* which were continuous. Several pairs of large, elongated, irregular bodies lie in the tail-stem on either side of the central excretory tubule which runs along the dorsal side of the tail-stem. Ordinarily two pairs of these glands are readily seen but in a few cases a third pair was noticed which was nearer the base of the stem. The sides of the stem are thrown into tiny irregular folds when the tail is slightly contracted, due to the circular muscle fibers in this region. Bundles of longitudinal fibers extend through the stem and into the furcae.

The oral sucker is elongated and oval in shape and measures $26\ \mu$ in length by $16\ \mu$ in width. Its narrow cavity opens into a short prepharynx which connects with a small bulbular pharynx. No other part of the digestive tract was observed.

A group of six penetration glands lies in the posterior half of the body but further anterior to the bladder than those of *C. hamata* and *C. multicellulata*. These glands are smaller and more easily broken under pressure than those of *C. bessaie* and similar forms, and cannot be detected easily even with the use of neutral red, since the entire body is literally packed with tiny, irregular or spherical nuclei which obscure all

structures in the body. Only the regions immediately lateral and posterior to the oral sucker are free from these nuclei. They make it very difficult to study the penetration glands and to determine their connections with their ducts. The glands differ from those of related forms in that those of a pair are usually not directly opposite but may be shifted anteriorly or posteriorly from such a position (Fig. 102). The ducts are bipartite and define a wavy course to the oral sucker. They narrow noticeably just before entering this organ, then swell to several times that of the posterior diameter. Each duct finally separates into two distinct parts just before opening at the anterior end of the worm in the region of a group of long spines.

A genital mass of small cells is just posterior to the penetration glands. When the bladder has emptied, two large globular cells can be seen in the posterior end of the body. Each contains a very large vesicular nucleus. Their function is unknown. No primordium of the acetabulum is evident.

The small excretory bladder has a lateral constriction which divides it into anterior and posterior parts. A caudal tubule extends along the median dorsal side of the tail and ends at a point exactly between the two furcae at the posterior margin. No branches have ever been seen in the furcae of the tail. The excretory pore opens on the dorsal side near the posterior end of the animal. The bladder gives rise to two main collecting tubules, each of which divides lateral to the anterior penetration glands. A posterior branch extends into the tail but flame cells were not seen in the stem. The anterior collecting tubule soon divides into two, the median one soon dividing again into two, an outer and an inner branch. The inner branch redivides and its mesial branch extends across the body and meets that from the opposite side to form a broad connecting loop just anterior to the mucin glands. The excretory system of this form differs in this respect from other members of this group but I am certain of the existence of this loop even though the terminal capillaries and cells of the excretory system were not traced.

This cercaria differs from all other described pharyngeal longifurcate monostomes in its possession of mucin glands of very indistinct character, small size, and uncertain position; an excretory system with at least one large anastomosing loop; the presence of two large globular cells near its posterior border; very numerous, closely packed body nuclei; and the existence near its furcal union of two discontinuous or broken rod-like structures differing from the solid ones of *C. hamata*. It also lacks the eye-spots of *C. physae*, *C. bessiae*, and *C. multicellulata*, the numerous body and tail spines of *C. dorsocauda*, the larger penetration glands of *C. hamata*, and the island of Cort of *C. rhabdocaeca*.

APHARYNGEAL LONGIFURCATE MONOSTOME CERCARIAE

Definition of the Group

A discussion of the apharyngeal brevifurcate distomes has been given previously. Those of the present group differ in having the furcae longer than one-half the tail-stem length, and in other features mentioned in Miller's description of the longifurcate forms. These forms have no ventral sucker.

Miller (1926a) found only one such form described in the literature up to that time, namely *Cercaria indica* xxvii Sewell 1922. One other form has more recently been described, *C. scwelli* Faust 1926.

During the present studies a member of this subgroup was found in *Physa gyrina* which was collected at Baton Rouge, Louisiana, in 1932. Morphological studies on this form enabled me to designate it as a new species in an earlier paper.

Cercaria louisiana E. L. Miller 1935

(Figs. 105-108)

Physa gyrina collected near Baton Rouge, Louisiana, in June, 1932, harbored an apharyngeal longifurcate monostome which I have designated as *Cercaria louisiana*. The worm is very active in the water, and when in contact with a substratum, turns its ventral side downward (Fig. 108). For this reason it is nearly always observed under the microscope with its dorsal side uppermost. The body contracts and extends slowly but the tail-stem has less power to do so. The hook shape, seen from side view and mentioned for many pharyngeal longifurcate monostomes, was not noticed for this form.

When well extended the body is as narrow as the tail-stem, with the exception of the very posterior end. The anterior end is bluntly rounded and bears on its surface a crown of large, narrow spines seen easily under low power. They reach posteriad for not quite one-third of the length of the oral sucker (Fig. 105). As the worm contracts large annulations appear which reach posteriad for about two-thirds of the body's length.

The body normally is a little shorter than the tail-stem, and the tail-stem and furcae are of about the same length. Under slight pressure the body is 0.168 mm. long, the tail-stem 0.224 mm., and the furcae 0.224 mm. At the same time the body is 43 μ wide, the tail-stem is 33 μ , and the furcae are 17 μ at their base. The stem does not taper at its distal end. Under great pressure the body is 0.196 mm. long but the tail-stem and furcae still measure 0.224 mm. in length. The body of the worm is slightly prolonged ventrally and this projection fits into the

tail's attachment (Fig. 105). A dorsal projection of the body covers the tail's attachment.

No caudal bodies are in the tail-stem. However, many nuclei are in both the stem and furcae (Fig. 106), and extending through the center of the tail is a broad band of muscle fibers. The band splits into two parts at the distal end of the stem and each part passes into a furca, reaching to its distal extremity. Also a horizontal band of fibers connects one furca with the other and fuses with the other band in each furca (Fig. 107). A narrow fin-fold is present on each furca. The delicate striations so commonly observed in the furcae of the pharyngeal forms are also present in this form, but the lines are less distinct, being more broken and fused than in other forms.

The terminal opening of the anterior organ opens into a narrow cavity lying between the swollen penetration ducts and extending through the sucker. No other part of the digestive tract was observed. The large, elongated, oval, oral sucker is about $59\ \mu$ long and $29\ \mu$ wide. No acetabulum is present but a packed group of small cells which lies between and slightly posterior to the two anterior penetration glands may represent the undeveloped ventral sucker.

Two dorsal, pigmented eye-spots are just in front of the anterior penetration glands. Each is a small, circular, compact body but pressure causes it to separate into a number of pigment masses. The eye-spots are ventral to the penetration ducts.

Six large penetration glands lie in the posterior region of the body, the anterior pair being just behind the small eye-spots. Their ducts extend forward as bipartite structures, the median half of each duct being filled with coarser granular material than that of the outer part of the duct. In the region of the oral sucker the ducts become very voluminous but narrow again just before opening on either side of the mouth opening near the dorsal side of the body. The inner part of each duct connects with an anterior gland while the outer part divides into two ducts each connecting with one of the two posterior glands.

Posterior to the penetration glands is a large mass of cells, the germinal primordium. Many deeply-staining nuclei cover the body's structures as they do the stem and furcae, but they are particularly dense in some regions of the body.

The lateral constrictions of the small excretory bladder are not pronounced. The excretory system was not studied further because the hosts did not live long enough to enable me to do so.

C. louisiana differs from *C. indica* xxvii in lacking the rhabdocoele gut and the numerous penetration glands of *C. indica* xxvii. Also the eye-spots of *C. indica* xxvii are non-pigmented, and its furcae are without the folds described for *C. louisiana*. *C. sewelli* differs from the above species in possessing a dorsal body keel, eye-spots with pigmented

centers and only two pairs of penetration glands. Both *C. furcalineata* and *C. bessiae* differ from *C. louisiana* in lacking the furcal fin-folds which it has.

X. CERCARIAEA

History and Definition

This name was first used by Diesing (1855) as a collective group name and not as a true generic name, for a group of little known larval trematode forms. Lühe (1909) defined the group as "Distomenlarven, bei welchen die Ausbildung eines Schwanzes überhaupt unterbleibt," and separated it into the two larval genera *Cercariaeum* s. str., and *Leucochloridium*.

Carus (1835) created the genus *Leucochloridium* to contain a species of larval trematode which he named *Leucochloridium paradoxum*, which Zeller found later developed into the adult *Distomum macrostomum*.

Since Carus' description of the first *Leucochloridium* from *Succinea amphibia* on an island in the Elbe, several of these larval forms have been described. However, *Leucochloridium* larvae have probably been discovered three times in North America up to this time. Call (1898) in speaking of *Succinea obliqua* in Indiana said, "the tentacles are rather large and thick, club-shaped, and are often the home of a stage in the development of a planarian." Ward (1918) states that Bryant Walker, in a personal letter, reported finding the larval stage of a *Leucochloridium* in *Succinea ovalis* in Michigan. Magath (1920) reported a larval form from Fairport, Iowa, and gave it the name *Leucochloridium problematicum*. He reported it from both *Succinea retusa* and *Planorbis trivolvis*. *L. assamense* was reported by Sewell (1922) from India, and *L. millsii* by Faust (1924) from China. *L. cercatus* has also been described by Monticelli.

The recent discovery by McIntosh (1927, 1932), in Michigan, of adult *Leucochloridium* has added significance in connection with the following second detailed report of the larval form from North America.

Leucochloridium problematicum Magath 1920

(Figs. 16-19)

On October 8, 1931, I collected two *Succinea ovalis* Say from smart weeds, above the water at Cole's Pond, Charleston, Illinois, and upon opening one of them I found a large capsule-like object in one of the tentacles which proved to be the large branch of a sporocyst inside the body of the snail. The proximal end is very narrow while the distal half

of the sporocyst is banded with deep golden or brownish-red bands, varying in hue from almost yellow to brown and reddish-brown.

This larva is remarkably like the one described by Magath (1920) at Fairport, Iowa, and which he called *Leucochloridium problematicum*. This larva also resembles the one first described by Carus (1835) which he named *L. paradoxum*. Of course the possibility arises that perhaps the green-banded sporocyst of Carus and the brown-banded ones which Magath and I have reported are one and the same larva. Later work may throw light on this subject. This sporocyst is about 12 mm. long in the living condition and tapers into a thin thread at its proximal end, while the distal end is narrowly rounded (Fig. 17). The wall of the sporocyst is very tough.

Small larvae could be seen inside the body. It is circled by reddish-brown bands at its distal end, there being four of them. Proximally the bands grow lighter, fading to a yellowish color, until, about midway between the two ends, two broad, reddish-brown bands again occur, being perhaps more brownish in color than the distal ones. Faint yellowish spots were also noted near the proximal end of the sporocyst. The color varies somewhat from that shown by Magath (1920) for *L. problematicum*; also the size is greater. However, it is very likely that his measurements were taken from preserved specimens while mine were made from the living worm. Nevertheless, the preserved sporocyst measured 6.0 mm. in length, which is still greater than Magath's measurements.

This sporocyst contained 147 small larvae, each enclosed in a relatively large, two-layered, transparent sac (Fig. 18), with the two suckers separated from the rest of the sac at their openings by a bulb-like, thin, transparent membrane (Fig. 19). The larvae twist about in their sacs, and in doing so they move the sacs about as though they were parts of the larvae's bodies. These sacs are about 1.37 mm. in length and about 0.823 mm. in width through their acetabular regions. The measurements were made from living worms without subjecting them to pressure. Some cysts are elongated and of a brownish color, some smaller and round and of a clear transparent color. The sporocyst was opened at 9:30 p.m. on the 13th of October and the larvae within were placed in salt solution. All of the cysts had partly dissolved by 11:00 a.m. on the 14th. The cysts about the worms which I fixed in warm Gilson's fluid also disintegrated.

The larvae are oval in shape, rounded at the anterior and narrower at the posterior end. When well extended they measure about 1.206 mm. in length and when contracted, about 0.767 mm. in length and 0.514 mm. in greatest width, taken through the region between the pharynx and acetabulum.

The oral sucker and acetabulum are large in proportion to the size of this tiny worm, the acetabulum lying near the anterior margin of the posterior third of the body. However, in the preserved specimen this is somewhat different, with the acetabulum at the anterior half of the body, as described by Magath. The suckers are both powerful, more so than they are pictured by Magath. The oral sucker is 0.297 mm. in length and 0.322 mm. in width when contracted, and about 0.3 mm. in length and 0.2 mm. in width when extended. It is situated about $56\ \mu$ posterior to the anterior end of the body when the body is extended. A very brief esophagus is evident when the worm is greatly extended; however, in the contracted, preserved individuals this cannot be seen. The pharynx in the living specimen is only slightly wider than long but in the preserved specimen measures $57\ \mu$ long and 0.124 mm. wide. In the living animal it is about 0.15 mm. long and 0.10 mm. wide when the body is extended. There is no prepharynx.

The two intestinal crura extend, in a wide arc, almost to the posterior end of the body (Fig. 16). In a contracted or preserved specimen they define a broad curve as they extend posteriad. The distance from the acetabulum to the posterior extremity of the body is about 0.277 mm. The large acetabulum is 0.24 mm. in diameter. It lies about 0.25 mm. back of the posterior end of the oral sucker, its anterior margin not reaching to the middle of the body. As mentioned previously, this position is greatly altered by preservation. The posterior ends of the ceca are slightly pointed and the thickened walls extend throughout their length.

The excretory bladder is relatively very small in proportion to the size of the worm and lies close to the posterior extremity of the body. It extends diagonally in a postero-dorsal direction where it opens through the excretory pore which is close to the posterior extremity of the body. Two powerful siphons extend antieriad, and lateral to the ceca to a point lateral to the oral sucker, then turn posteriad and at a point just anterior to the bladder divide into several secondary branches. The largest branch extends antieriad, giving off branches in its course, until it ends as a single fine collecting tubule lateral to the oral sucker. Each capillary ends in a flame cell but because of the intricate network of vessels and flame cells (Fig. 16) it was impossible to be certain of the connections of all parts of this complicated excretory system. Material on hand was limited.

The genital organs are well developed in these larvae; the two testes, the ovary, the rounded organ, and cirrus sac that Magath (1920) mentions for *L. problematicum* are all readily identified. The genital opening is on the dorsal side of the body and posterior to the reproductive organs. It is near the posterior extremity of the body, even posterior to the excretory pore.

The locations of these reproductive glands vary in the preserved and mounted specimens. It should be noted that the testes are described here as elongate ovals rather than spheres, as they have been pictured in drawings of *L. problematicum* and *L. paradoxum*. Measurements of these organs in preserved specimens were taken. The anterior testis is to the right of the midline and is $67\ \mu$ long; the left posterior testis is $57\ \mu$ long; the spherical ovary is on the left side of the body and is $52\ \mu$ long and $48\ \mu$ wide; the elongated cirrus sac tapers anteriorly and is $76\ \mu$ by $48\ \mu$; the so-called round body, lies between the two testes, and it is $48\ \mu$ in diameter.

In spite of several differences between these forms under discussion, I shall not designate this form as a new species, since work is being continued regarding its life history and morphology. With the present evidence, I am inclined to refer to it as only the first report of *Leucochloridium problematicum* from Illinois, and the second report from North America.

XI. SUMMARY

1. The larval trematode fauna of areas of Illinois, Louisiana, and Florida has been studied.
2. Nineteen previously described species from Illinois, Iowa, Michigan, Louisiana, Florida, and Wisconsin have been studied.
3. Four new species have been added to the list of North American forms, one from Florida, two from Louisiana, and one from both Illinois and Louisiana.
4. All descriptions were made from the living animal under uniform conditions.
5. Each species has been placed in its proper group, the history and definitions for each group having been previously given.
6. The twenty-three larvae are one Cercariaeum, two Gorgoderines, one Monostome, one Echinostome, one Cercaria Ornata, three Cercariae Microcotylae, six Cercariae Armatae, three Apharyngeal Brevifurcate Distome Furcocercous Cercariae, four Pharyngeal Longifurcate Distome Furcocercous Cercariae, and one Apharyngeal Longifurcate Monostome Furcocercous Cercaria.
7. Several new hosts and many new localities were found for previously described cercariae.
8. Complete infection records for all forms have been correlated in tables.
9. An identification key for all described Illinois larval trematodes has been prepared.
10. The relative merits of various morphological features of the larva for diagnostic value have been discussed.

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EXPLANATION OF PLATES

Unless otherwise stated, all drawings were made from living material under pressure of the cover-slip, with the aid of a camera lucida. Outlines, and in most cases, the suckers, were drawn with camera lucida and the remaining structures inserted free-hand. In all cases the penetration glands were drawn after neutral red had been added.

ABBREVIATIONS

<i>a</i> anterior (penetration) organ	<i>g</i> gelatinous cyst
<i>ac</i> anterior cell mass	<i>gb</i> germ ball
<i>av</i> anterior vesicle	<i>gc</i> germ cell-mass
<i>b</i> folded body	<i>i</i> island of Cort
<i>bp</i> birth pore	<i>l</i> longitudinal muscles
<i>c</i> covering cells of bladder	<i>lv</i> lateral vesicle
<i>ca</i> capillary	<i>mc</i> main collecting tubule
<i>cb</i> caudal body	<i>me</i> median eye-spot
<i>cc</i> cyst cavity	<i>o</i> ovary
<i>cg</i> cystogenous gland	<i>pe</i> pigmented eye-spot
<i>cl</i> caudal lumen	<i>pg</i> penetration gland
<i>cn</i> caudal nucleus	<i>pl</i> posterior locomotor appendage
<i>cp</i> constriction	<i>pm</i> posterior mucin body
<i>cpo</i> caudal pocket	<i>pv</i> posterior vesicle
<i>cs</i> cirrus sac	<i>rb</i> round body
<i>ct</i> globe cavity	<i>s</i> swollen ridge
<i>d</i> duct of penetration glands	<i>sc</i> secondary collecting tubule
<i>e</i> encysted individual	<i>sh</i> sensory hairs
<i>eb</i> excretory bladder	<i>sm</i> sphincter muscle
<i>eg</i> excretory granules	<i>t</i> testis
<i>f</i> fin-fold	<i>tf</i> tail folds
<i>fr</i> furcal rays	<i>tg</i> tail globe
<i>fro</i> furcal rod	<i>tt</i> thread of tail
<i>fs</i> furcal striations	<i>vc</i> vesicular cell

PLATE I

Note.—Fig. 8 is semi-diagrammatic; the scale for Fig. 6 represents 0.01 mm.; scales for Figs. 1, 2, 5, 13, and 14 represent 0.05 mm.; scales for all other figures represent 0.1 mm.

FIGS. 1-9.—*Cercaria sphaerocerca*

- FIG. 1.—Ventral view, digestive system shown in broken lines to denote position in body; suckers semi-diagrammatic.
FIG. 2.—Structure of tail globe under pressure.
FIG. 3.—Encysted worm in proximal cavity of tail globe.
FIG. 4.—Entire tail appendage showing three distinct parts.
FIG. 5.—Side view showing prominence of ventral sucker.
FIG. 6.—Side view of stylet showing its dorsal hump.
FIG. 7.—Distribution of nuclei in distal portion of tail as revealed by neutral red.
FIG. 8.—Various shapes assumed by the bladder during life; semi-diagrammatic.
FIG. 9.—Sporocyst showing undeveloped body on tail globe within.

FIGS. 10-15.—*Cercaria mitocerca*

- FIG. 10.—Ventral view of body showing main features.
FIG. 11.—Ventral view showing spherical cell-like bodies after collapse of excretory bladder under pressure.
FIG. 12.—Tail appendage showing three definite divisions.
FIG. 13.—Proximal vesicle of tail globe showing thick walls.
FIG. 14.—Surface of tail globe during contraction showing fissures.
FIG. 15.—Sporocyst showing typical shape.

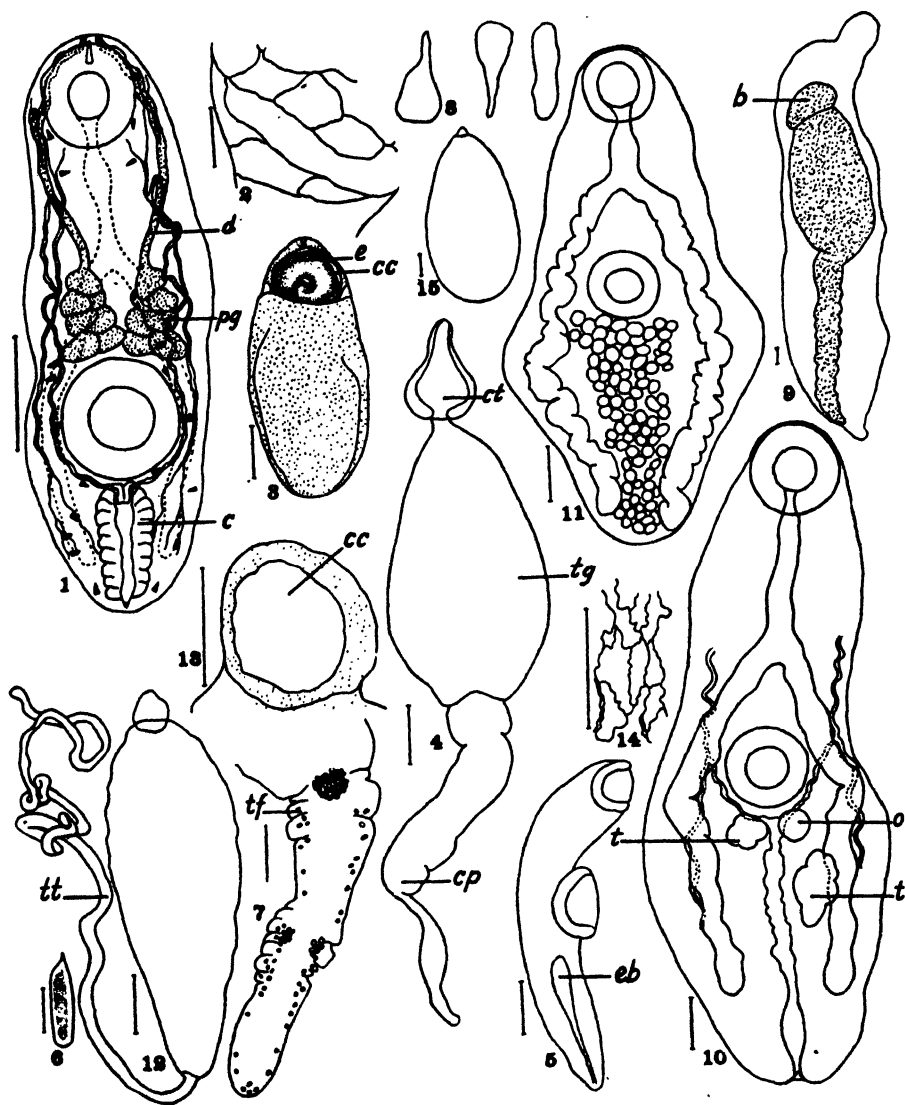


PLATE I

PLATE II

Note.—Figs. 19, 31, and 32 are semi-diagrammatic drawings; scales for all other figures represent 0.1 mm.

FIGS. 16-19.—*Leucochloridium problematicum*

- FIG. 16.—Ventral view, showing principle branches of excretory system.
FIG. 17.—Banded sporocyst from the snail's tentacle.
FIG. 18.—Body together with gelatinous sheath surrounding it, while subjected to strong pressure.
FIG. 19.—Side view of same showing connections of suckers to outside.

FIGS. 20-27.—*Cercaria urbanensis*

- FIG. 20.—Dorsal view, showing main structures of body.
FIG. 21.—Early stage of encystment immediately after loss of the tail.
FIG. 22.—Much later stage of encystment still showing region of tail attachment.
FIG. 23.—The worm about forty-eight hours after encystment.
FIG. 24.—Redia showing complete digestive cecum.
FIG. 25.—Characteristic shape of old redia.
FIG. 26.—Young redia with cercariae in various stages of development.
FIG. 27.—Cercaria in an early stage of development; taken from redia.

FIGS. 28-32.—*Cercaria trivolvis*

- FIG. 28.—Dorsal view of the worm with excretory tubules and cystogenous glands shown on alternate sides.
FIG. 29.—Mature redia showing digestive cecum and birth pore.
FIG. 30.—Redia showing posterior locomotor appendages and anterior collar.
FIG. 31.—End of tail showing the small distal body only partially invaginated; semi-diagrammatic.
FIG. 32.—End of tail showing the distal body protruding completely; semi-diagrammatic.

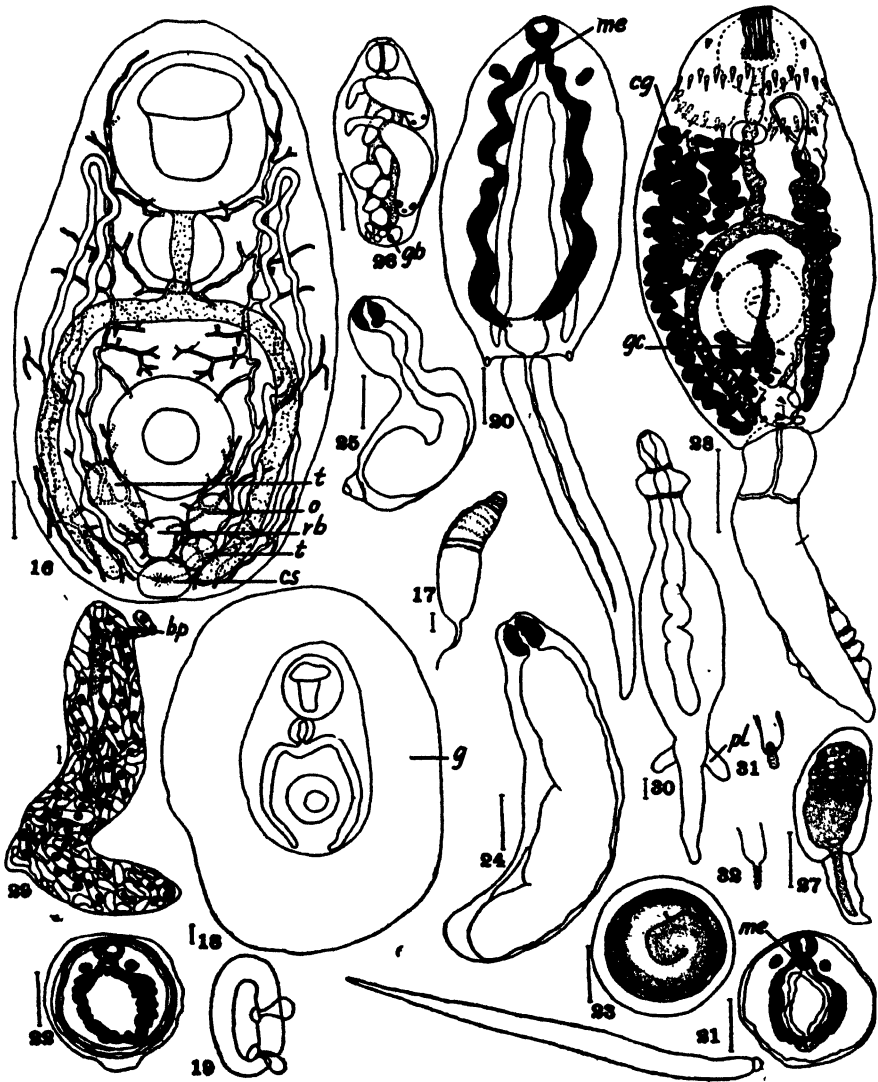


PLATE II

PLATE III

Note.—Figs. 34 and 48 are semi-diagrammatic drawings; scales for Figs. 39, 43, and 44 represent 0.01 mm.; scales for Figs. 41, 42, 47, 49, and 52 represent 0.05 mm.; scales for all other figures represent 0.1 mm.

FIGS. 33-34.—*Cercaria trivolvis*

FIG. 33.—Redia showing posterior appendages and withdrawn anterior end.

FIG. 34.—Nuclei of tail under very strong pressure.

FIGS. 35-40.—*Cercaria mesotylpha*

FIG. 35.—Ventral view of body.

FIG. 36.—Ventral view showing complete excretory system.

FIG. 37.—Mass of sporocysts in the liver tissue.

FIG. 38.—One end of a sporocyst enlarged to show cercariae within.

FIG. 39.—Dorsal view of stylet, showing its globular base.

FIG. 40.—Sagittal section showing germinal masses dorsal to acetabulum.

FIGS. 41-46.—*Cercaria cystorhysa*

FIG. 41.—Ventral view of body; dorsally located stylet shown with solid outline; body contracted.

FIG. 42.—Ventral view showing main branches of excretory system; extended.

FIG. 43.—Dorsal view of stylet, showing lateral shoulders.

FIG. 44.—Tail under strong pressure and neutral red, showing nuclei and caudal hairs.

FIG. 45.—Sporocyst containing cercariae in various stages of development.

FIG. 46.—Mass of sporocysts attached to the liver tissue of the snail.

FIGS. 47-51.—*Cercaria meniscadena*

FIG. 47.—Ventral view with principle structures shown.

FIG. 48.—Contracted tail showing lateral folds; semi-diagrammatic.

FIG. 49.—Sporocyst with developing cercariae.

FIG. 50.—Sporocyst showing characteristic knobs of older forms.

FIG. 51.—Sporocyst with more pronounced knob.

FIG. 52.—*Cercaria cyclica*

FIG. 52.—Ventral view of the body showing penetration apparatus.

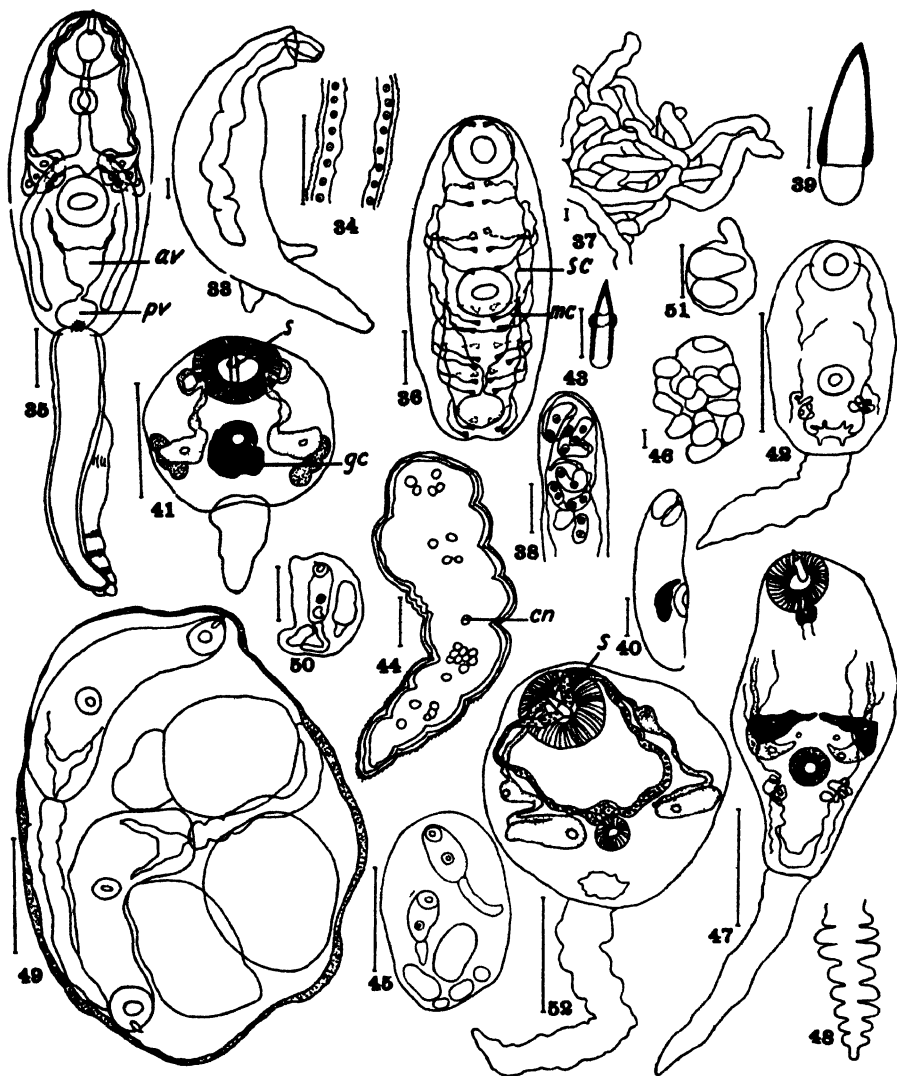


PLATE III

PLATE IV

Note.—Fig. 57 is a semi-diagrammatic drawing; scales for Figs. 56, 62, 63, and 67 represent 0.01 mm.; scales for Figs. 55, 58, 61, 66, and 68 represent 0.1 mm.; scales for all other figures represent 0.05 mm.

FIG. 53.—*Cercaria cyclica*

FIG. 53.—Ventral view showing main tubules of excretory system.

FIGS. 54-59.—*Cercaria acanthocoela*

FIG. 54.—Ventral view of body.

FIG. 55.—Lateral view of body under no pressure.

FIG. 56.—Dorsal view of stylet showing lateral projections.

FIG. 57.—Various shapes assumed by excretory bladder during life; semi-diagrammatic.

FIG. 58.—Sporocyst showing typical shape; cercariae and germ balls within.

FIG. 59.—Encysted individual showing distension of bladder by granules.

FIGS. 60-65.—*Cercaria tricystica*

FIG. 60.—Dorsal view of body, with suckers shown in broken lines.

FIG. 61.—Ventral view with penetration ducts showing.

FIG. 62.—Dorsal view of stylet showing soft base.

FIG. 63.—Lateral view of same stylet.

FIG. 64.—Tail treated with neutral red to show nuclei.

FIG. 65.—Sporocyst with typical elongate-oval shape.

FIGS. 66-68.—*Cercaria cytonchnoides*

FIG. 66.—Contracted tail showing central lumen.

FIG. 67.—Dorsal view of stylet showing lateral swellings.

FIG. 68.—Lateral view of body when greatly extended during movement; very little pressure.

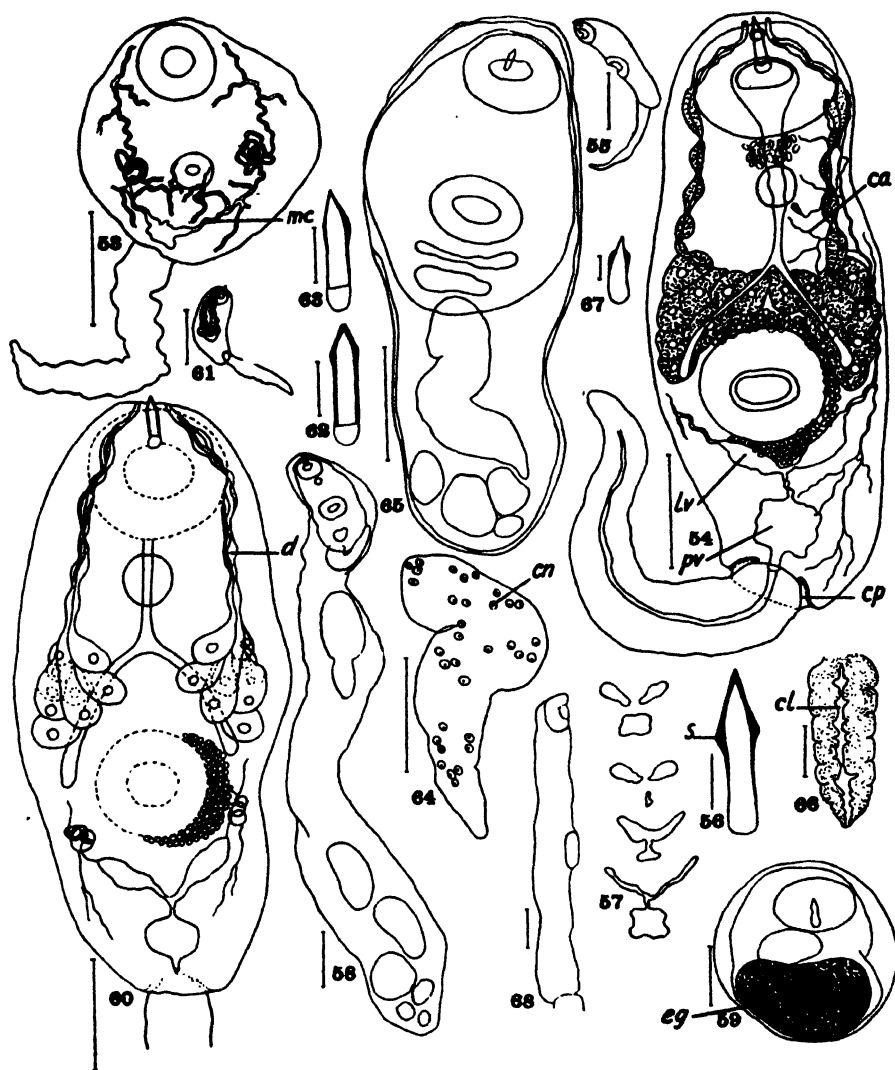


PLATE IV

PLATE V

Note.—Scales of Figs. 72 and 76 represent 0.01 mm.; scales for Figs. 71 and 74 represent 0.05 mm.; scales for all other figures represent 0.1 mm.

FIGS. 69-70.—*Cercaria cystonchnoides*

FIG. 69.—Dorsal view of body showing complete excretory system.

FIG. 70.—Sporocyst showing typical terminal knobs.

FIGS. 71-73.—*Cercaria steganocoela*

FIG. 71.—Dorsal view of body.

FIG. 72.—View of stylet when turned slightly on its side.

FIG. 73.—Drawing showing filiform sporocyst with various stages of cercariae within.

FIGS. 74-76.—*Cercaria pachycystata*

FIG. 74.—Dorsal view of the body.

FIG. 75.—Mass of sporocysts in the liver tissue.

FIG. 76.—Top view of stylet, showing soft, globular base.

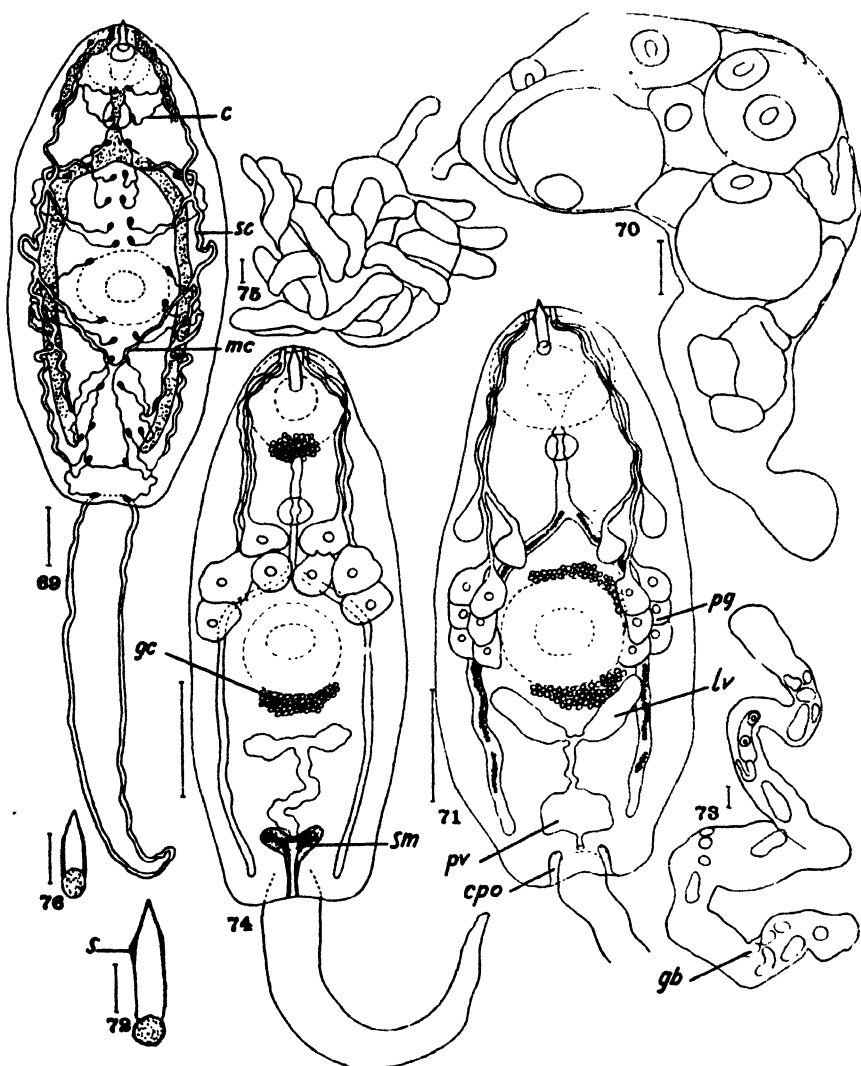


PLATE V

PLATE VI

Note.—The scale for Fig. 87 represents 0.01 mm.; scales for Figs. 79, 80, 82, 85, and 88 represent 0.1 mm.; scales for all other figures represent 0.05 mm.

FIG. 77.—*Cercaria pachycystata*

FIG. 77.—Dorsal view of body to show branches of excretory system in posterior regions of the body.

FIGS. 78-80.—*Cercaria tridena*

FIG. 78.—Dorsal view of the body.

FIG. 79.—Lateral view of the body under no pressure.

FIG. 80.—Sporocyst showing one encysted individual.

FIGS. 81-87.—*Cercaria wardi*

FIG. 81.—Lateral view of the body with the eye-spots flattened by pressure.

FIG. 82.—Tail, showing fin-folds, striations in furcae, and caudal excretory tubule.

FIG. 83.—Furca, showing rayed fin-folds in their dorsal and ventral positions.

FIG. 84.—Lateral view of body, showing complete excretory system.

FIG. 85.—Section of a sporocyst showing developing germ balls and cercariae.

FIG. 86.—Anterior end of body showing dorsal view; also showing spined crown, pyriform anterior organ, digestive tract, and eyes.

FIG. 87.—Greatly magnified view of a caudal flame cell immediately after movement has ceased, showing striations.

FIG. 88.—*Cercaria pteractinota*

FIG. 88.—Dorsal view of body showing eye-spots and the large branches of the excretory system.

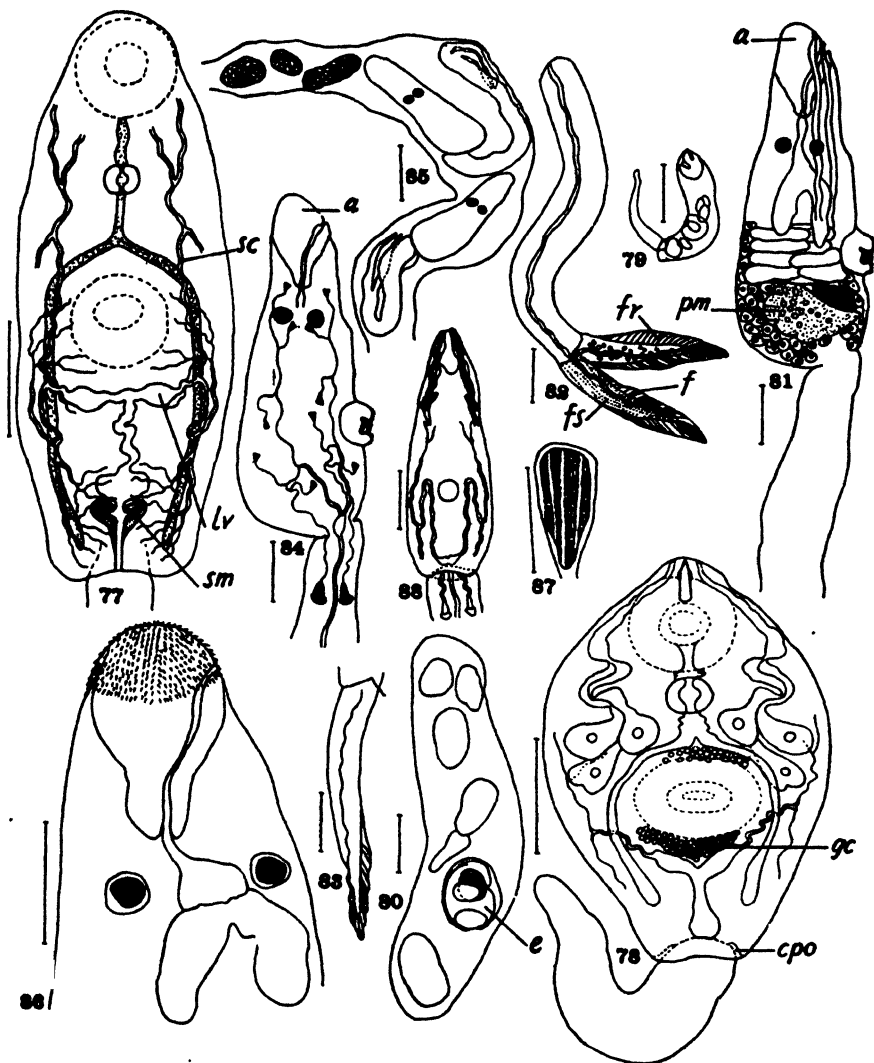


PLATE VI

PLATE VII

Note.—Fig. 94 after original by Faust; Fig. 99 after original by H. M. Miller; scales for Figs. 89, 92, and 98 represent 0.1 mm.; scales for all other figures represent 0.05 mm.

FIGS. 89-93.—*Cercaria pteractinota*

FIG. 89.—Portion of sporocyst showing developing cercariae and germ balls.

FIG. 90.—Furca, showing the rays of the fin-fold.

FIG. 91.—Dorsal view of body showing eyes and the other six principle pigment masses; other pigment not shown. Also the pyriform sucker and crown of spines are shown.

FIG. 92.—Dorsal view showing the entire cercaria.

FIG. 93.—Lateral view to show position of numerous penetration glands and the germinal masses.

FIG. 94.—*Cercaria gigas*

FIG. 94.—Dorsal view of cercaria; after original of Faust; $\times 125$

FIGS. 95-98.—*Cercaria hamata*

FIG. 95.—Dorsal view of body, showing penetration glands, germ masses, digestive tract and complete excretory system.

FIG. 96.—Tail-stem showing sensory hairs and caudal bodies.

FIG. 97.—Furca showing central lumen and small furcal rod.

FIG. 98.—View of cercaria, showing relations of body, tail-stem, and furcae.

FIG. 99.—*Cercaria multicellulata*

FIG. 99.—Dorsal view; after original of Miller; $\times 500$

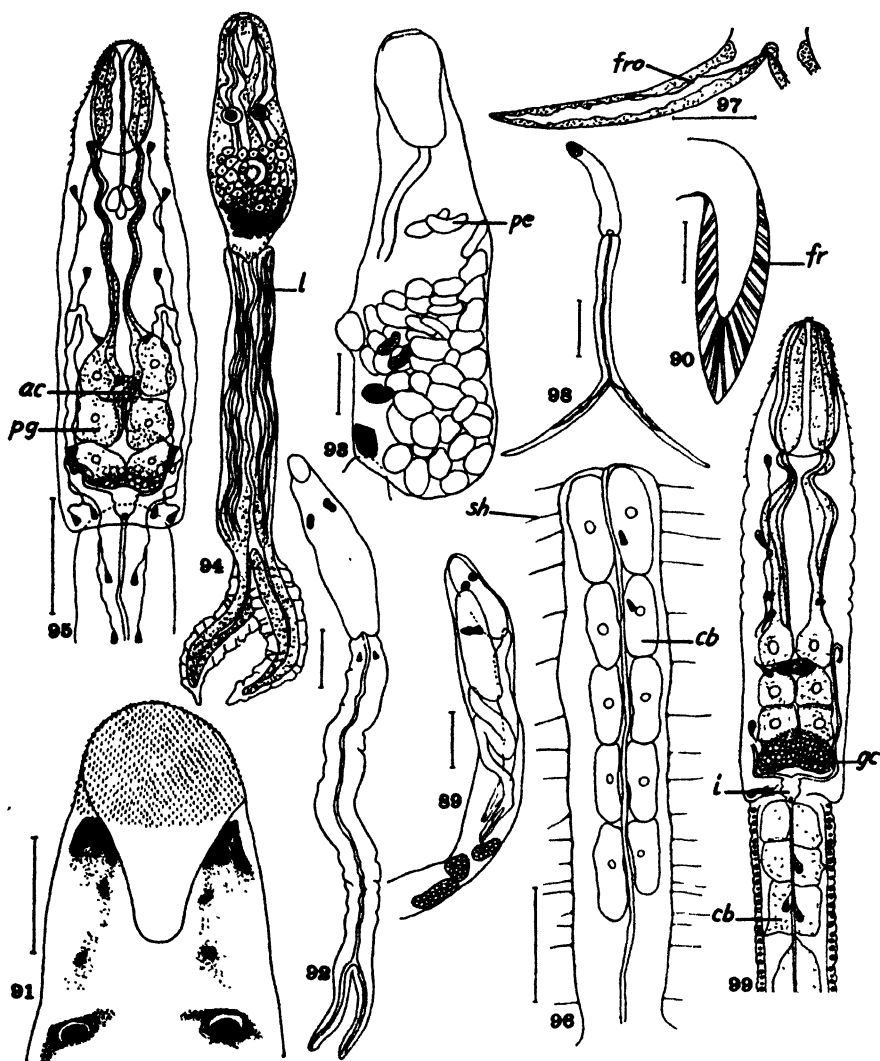


PLATE VII

PLATE VIII

Note.—Fig. 100 after original by Cort and Brooks; scales for Figs. 101, 103, and 108 represent 0.1 mm.; scales for all other figures represent 0.05 mm.

FIGS. 100-101.—*Cercaria bessiae*

FIG. 100.—Dorsal view of *Cercaria*; after original of Cort and Brooks; semi-diagrammatic drawing.

FIG. 101.—Section of filiform sporocyst.

FIGS. 102-104.—*Cercaria furcalineata*

FIG. 102.—Dorsal view of body and anterior portion of tail-stem.

FIG. 103.—Entire cercaria without pressure, to show relation of body, tail-stem, and furcae.

FIG. 104.—Enlarged furca showing furcal striations and position of nuclei.

FIGS. 105-108.—*Cercaria louisiana*

FIG. 105.—Dorsal view of body showing anterior crown of spines, oval anterior organ, eye-spots, penetration glands, germinal mass, rudimentary sucker, and excretory bladder.

FIG. 106.—Tail-stem, showing position of muscle bands and nuclei after neutral red has been added.

FIG. 107.—Furca, showing fin-folds, nuclei, and position of muscle bands.

FIG. 108.—Entire cercaria showing relations of body, tail-stem, and furcae; drawn without the application of pressure.

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**STUDIES ON THE MORPHOLOGY AND
LIFE HISTORY OF NEMATODES IN
THE GENUS SPIRONOURA**

WITH FIVE PLATES AND TWO TEXT-FIGURES

**By
JOHN GILMAN MACKIN**

**CONTRIBUTION FROM THE ZOOLOGICAL LABORATORY OF THE
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I. INTRODUCTION

Few extensive studies have been made on the anatomy and growth variations of nematodes, and these have been confined so largely to isolated and economic forms that nothing like a comprehensive knowledge of the group as a whole is available. Thus it has been deemed worth while to extend our knowledge through study of a form not too nearly related to the Ascarids, Oxyurids, and Ancylostomas, already well known.

Extensive collections of several species of the genus *Spironoura* have provided sufficient material to make such studies possible. Species of this genus are almost constant inhabitants of the posterior alimentary canal of various species of turtles. Most of the collections were made in Oklahoma.

Because I have come to the conclusion that the separation of families, subfamilies, and genera should depend primarily upon the variations in the digestive and reproductive systems, I have placed greatest emphasis on the study of these two systems. When speaking of the reproductive system, I mean the genital tubes. I believe that male secondary organs, such as spicules, gubernaculum, and caudal papillae have been made to bear a disproportionately great part in diagnoses of groups of these ranks. Until general knowledge of fundamental types of the major systems is made available, our taxonomic schemes must remain unnatural and more or less makeshift.

In addition to the structural studies, taxonomic studies have been made on the North American species of *Spironoura*, and a key compiled for ready determination.

I am indebted to Dr. Henry B. Ward for much encouragement and criticism during the work, and take this opportunity to express my appreciation. I also am indebted to him for the loan of slides and specimens, and for the use of his extensive personal library.

II. ANATOMY

Spironoura chelydrae (Harwood) 1932

GENERAL EXTERNAL MORPHOLOGY

The genus *Spironoura* belongs in the family Kathlaniidae Travassos, and to the subfamily Kathlaniinae. The family is one of the oxyuroid groups containing reptilian parasites for the most part and including several species parasitic in fishes and amphibians. *Spironouran* species are found parasitic in representatives of all three groups, but are primarily

and characteristically parasitic in turtles. From North America at the present time eight species have been described, and two more to be described in this paper bring the total number of species to ten. Each of these will be discussed later.

The species *S. chelydrae* was chosen for an extended anatomical study for several reasons. I believe the species to be representative of one of those groups which have become parasitic only recently, relatively speaking, as evidenced by the position within the host (rectum) which is close to one of the portals of entry. The medium within which these worms live is not so very different from the habitat of some free-living species which live in fresh or decaying dung. Specimens of *Spironoura* live for the most part in the feces of the host and feed upon this material, as evidenced by studies of the intestinal contents. Moreover, anatomically the group is not far distant from *Rhabditidae*, a family composed of both free-living and parasitic species.

The species was described by Harwood (1932). His description is rather brief, and the single drawing (of the tail of a male) decidedly sketchy. He placed the species in the genus *Falcaustra* Lane, which I consider a synonym of *Spironoura*.

MORPHOLOGY.—The form of the worms may be seen in Figs. 3 and 12. Specimens fixed in hot fluids never straighten entirely, because in the male there are accessory ventral muscles, and in the female the ventral somatic muscles are a little wider than in the dorsal sectors. Thus in the male the "lateral" lines are somewhat dorso-lateral. The body tapers to a point posteriorly and anteriorly; the head, however, although much smaller in diameter than the middle region, is bluntly truncate (Fig. 20). The female is distinctly narrower posterior to the vulva than anteriorly. Only a small portion of the reproductive system extends posterior to the vulva. The tail in both sexes is sharply pointed and is shaped like a narrow cone. In the female it is apt to be nearly straight; in the male it is always more or less curved ventrally in fixed specimens, the amount of curvature depending upon the temperature of the fixative. Some specimens may be merely curved as in Fig. 12, while others are spirally coiled with two or three loops. Complete measurements are given in Tables 1 and 4.

A cross section in the middle trunk region is not circular. The worms in the middle region of the body have a greater dorso-ventral diameter than transverse, being laterally flattened. This may be an effect of fixation, but it is constant in all specimens I have sectioned.

In the male, just anterior to the anus is a pair of longitudinal trough-like depressions (Fig. 10). The median preanal papilla is situated on a narrow ridge separating the two grooves. This is apparently a specific

character, since it is not found in other species. In the female, only a single depression is present in this region, the separating ridge being absent.

The head bears three low lips, one dorsal, the other two subventral (Figs. 17 and 20). There are four papillae to each lip, two internal and two external. All of these are of the sessile type.

In addition to the papillae the two subventral lips each bear an amphid (Fig. 17). These structures have not been hitherto reported for *Spiro-noura*. They are situated laterally, each very close to one of the papillae. In anterior view they appear as smooth convex circular areas, with a transverse slit across the middle. They are observable in ventral views of the head also (Figs. 20, 60, 64, etc.).

The cervical papillae are situated laterally, far back from the head (1.1 mm.) just anterior to the level of the anterior end of the excretory bridge.

The male has 10 pairs of caudal papillae and an unpaired median preanal papilla. The arrangement of these is shown in Fig. 10. Two pairs are lateral, both postanal, one immediately posterior to the level of the anus, and one farther back (numbers 3 and 4, respectively). The remainder are subventral. Two pairs of these are postanal, situated near the level of the posterior lateral pair (Fig. 10, numbers 1 and 2). Three pairs are circum-anal (numbers 5, 6, and 7). Usually all of these are

TABLE 1.—MEASUREMENTS OF APPROXIMATELY MAXIMUM AND MINIMUM MATURE SPECIMENS (IN MILLIMETERS)

Spiro-noura chelydrae (Harwood) 1932

Females	Mini- mum	Maxi- mum	Males	Mini- mum	Maxi- mum
Total length.....	9.5	22.55	Total length.....	6.65	16.3
Greatest breadth.....	0.47	0.89	Greatest breadth.....	0.32	0.66
Head breadth.....	0.09	0.18	Head breadth.....	0.08	0.16
Breadth at anus.....	0.20	0.47	Breadth at anus.....	0.18	0.46
Pharynx length.....	0.08	0.10	Pharynx length.....	0.07	0.10
Pharynx breadth.....	0.08	0.12	Pharynx breadth.....	0.06	0.10
Cylindric esophagus length..	1.24	1.84	Cylindric esophagus length	1.60	1.79
Cylindric esophagus breadth	0.10	0.15	Cylindric esophagus breadth	0.08	0.13
Anterior bulb length.....	0.18	0.30	Anterior bulb length.....	0.13	0.25
Anterior bulb breadth.....	0.10	0.18	Anterior bulb breadth.....	0.08	0.17
Posterior bulb length.....	0.17	0.29	Posterior bulb length.....	0.14	0.26
Posterior bulb breadth.....	0.18	0.29	Posterior bulb breadth....	0.16	0.27
To excretory pore.....	1.22	1.98	To excretory pore.....	0.86	1.82
To nerve ring.....	0.34	0.47	To nerve ring.....	0.28	0.46
Rectum length.....	0.20	0.53	Cloaca length.....	0.18	0.49
To vulva.....	6.23	13.68	Sucker to anus.....	1.87	4.60
Vagina length.....	2.44	4.82	Spicule length.....	2.35	4.50
Tail length.....	0.66	1.64	Tail length.....	0.40	1.00
Eggs length.....	0.099	0.105	Gubernaculum length.....	0.14	0.20
Eggs breadth.....	0.059	0.072			

slightly posterior to the anus, but one of them is sometimes anterior to the level of the anus. It seems better, therefore, to designate these as circum-anal to avoid confusion resulting from variability. The last three pairs are preanal. One of these is situated at about the level of the anterior end of the cloaca (number 8), the next pair 0.26 mm. anterior to this one, and the last pair 0.35 mm. anterior to the latter in an 11-mm. male (numbers 9 and 10 not shown in Fig. 10).

While the oblique muscles of the male are not external structures they are visible even in some uncleaned specimens and should be mentioned. The obliques begin just anterior to the anus and extend forward about a fourth of the body length. The pseudo-sucker is similarly situated just anterior to the obliques, and consists of a pair of fan-shaped muscular areas.

LIP SUPPORTS

Special consideration is given to the lip supports because they have been so generally misrepresented, because they afford such a definite character binding the various species of the genus *Spironoura*, and because in variational details they afford characters of specific value. In following the description, reference should be made to Figs. 17, 20, 57, etc.

The support is generally said to be cuticular, which is correct only if taken to mean that it is composed of a hard, homogeneous substance, non-nucleated and perhaps of the nature of a secretion. If the term *cuticular* is taken to mean that the support is of the same substance as the body-wall cuticula, it is probably used incorrectly. There is reason to believe that several so-called cuticular structures, such as the gubernaculum, the spicules, and the lip support, which are internal and not derived from the proctodeum or stomodeum, are composed of an entirely different substance from the body-wall cuticula. The data from staining reactions tend to support this view.

The ring support of the lips, as the name implies, is a ring composed of a hard substance encircling the lips; it affords an immovable point of origin for the muscles of the lips, as well as for parts of the anterior somatic musculature, the pharyngeal muscles, and the papillae tubes; and it serves to bind together the angles of the lips, this function being perhaps the least significant of all. The structure is not a thickening of the head cuticula, but rather an internal structure in its entirety (Fig. 52), a point on which several authors have been in some confusion (Walton, 1930).

The "ring" is not circular but tends to be more or less hexagonal. Each angle of the hexagon is modified and thickened. I call these thickenings the *nodes* of the support. There are two types of nodes: those found at the angles of the triangular vestibule, and those in the middle

region of each lip. The latter I refer to as the *pharyngeal supports*, from their principal function, and the former as the *angle supports*, from their position. Between each angle support and the adjacent pharyngeal support on each side, there are flattened bars, somewhat curved inwardly, to which I refer as the *connecting bars*.

Each angle support consists of an irregular piece (Figs. 17, 20, and 57) curving somewhat around the angles of the vestibule, with connecting bars projecting laterally on each side to the pharyngeal supports, and with a single triangular piece projecting posteriorly which makes contact with the angle of the pharyngeal cuticula lying in the same radius. This latter piece is specifically characteristic in surface view (Fig. 20). Muscle attachments to the angle supports consist only of fibres from the somatic muscles.

Each pharyngeal support lies in the center of a lip. It consists of a flattened anterior piece, with the lateral connecting bars projecting on each side, and a posterior piece in which the origins of some of the anterior pharyngeal muscles are found. The anterior plate affords a firm point of origin for the lip muscles (Fig. 52) which are attached in a row to the anterior surface, and spread fan-wise to the internal and anterior surfaces of the lips. Lip movement is accomplished by these muscles. The anterior plate also affords firm attachment for fibres from the somatic muscles. The posterior part of the pharyngeal support fits against the posterior surface of the anterior plate. Its own posterior and internal surface is deeply concave to accommodate the rounded surface of the anterior radial muscles of the pharynx which have their origin in it. The pair of backwardly projecting arms (formed by the posterior concavity) connect with the tunica propria of the pharynx.

To the connecting bars are attached the tubes of the papillae, which pass around the bars on the outside, lying in the concavities between nodes.

I believe it is clear that the type of support described by Baylis and Daubney for the genus *Zanclophorus* is closely related to that of *Spiro-noura*, if not identical with it. The horseshoe-shaped supports for the angles of the lips described by them are undoubtedly what I refer to in this paper as the angle supports. I should be very much surprised if further investigation does not reveal that the pharyngeal supports and connecting bars are present in *Zanclophorus* also. I might point out that *S. chelydrae*, were it not for the nature of the lip supports and papillae, would be practically identical in all other generic characters to *Zanclophorus*. The only North American species referred to *Zanclophorus* is Walton's *Z. cryptobranchi*, which I have determined by study of the type specimens is an undoubted *Spiro-nouran*. Since I prefer to limit my

studies as much as possible to North American species, I do not feel justified in making any final conclusion concerning the validity of the genus *Zanclophorus*.

A note concerning the structure of the median strands of the lateral lines may be inserted here. These structures (Figs. 16, 18, 19) are certainly not of the same tissue as the lateral strands. There are several evident differences. First, the nuclei are larger and much less numerous in the median strands and form a single row down the length of the body. Secondly, the cytoplasm has marked dissimilarities as shown by staining reactions. Lastly, the median strand is not a syncytium. The cell boundaries, as definite walls, are clearly depicted in tangential sections. The cell shape is that of an oblong rectangle, with the long axis coinciding with that of the body. The strands are each composed of a single row of cells. Some free-living nematodes have been found to correspond to this condition, which I take to be primitive.

DIGESTIVE SYSTEM

In *Spironoura* the digestive system consists of five parts. These are, naming from anterior to posterior, (1) vestibule, (2) pharynx, (3) esophagus, (4) intestine, and (5) proctodeum (Fig. 3). I am using the nomenclature of Yorke and Maplestone (1926) in this paper.

The vestibule is that portion of the digestive tract bounded by the lips (Figs. 17 and 52). A cross section has the shape of an equilateral triangle. Its depth is shallow, about 20 to 30 μ , depending somewhat on the state of contraction of the lips. The posterior boundary is the anterior surface of the muscles of the pharynx. The cuticula of the vestibule is not, however, continuous into the pharynx, and staining reactions indicate that the lining in the two parts is of somewhat different substance. The anterior edges of the three lips, which form the anterior boundaries of the vestibule, bear thickened transverse ridges, wider in the center of the lips. In longitudinal section these appear as blunt lobes (Fig. 52). Just posterior to these ridges is a second similar set of transverse ridges, rather more sharply edged than the anterior ones. These are the structures, illustrated in many figures of the head of species of *Spironoura*, that appear to be inwardly directed triangular points set in the middle of each lip. They actually represent optical sections of the ridges. The relative prominence of the ridges and the angle assumed in relation to the longitudinal axis of the body depend on the degree of contraction of the lips.

The pharynx is a short region bounded anteriorly by the vestibule and posteriorly by the esophagus (Figs. 20 and 47). The total length is about 0.08 mm., measuring the length of the muscles, and the width (ventral

view) is 0.75 mm. The pharynx is distinctly different, both as to lining and musculature, from the esophageal region.

The lumen, often spoken of as wide or narrow, is wide or narrow in proportion to the degree of contraction of the muscles. The breadth of the lumen is not, therefore, a taxonomic character, although given in some generic and specific diagnoses. A cross section is triangular or tri-radiate, the angles of the lumen are bluntly acute, and the middle of each flat surface has an inwardly projecting blunt ridge, running the length of the pharynx. The shape of the lumen is shown in Fig. 47.

The lining is thick and obviously composed of two layers. At the angles of the lumen the two layers are discrete, since they must slide upon each other when the pharynx is opened or closed. The primary or inner layer is thinner than the secondary layer, and is continuous all the way around, while the secondary layer is interrupted in the middle of each muscle field, allowing insertion of the large surface muscles to the primary cuticula. The median ridges are produced anteriorly into the basal region of the vestibule, not as sharp teeth, but as flat plates, to form a grinding organ. In longitudinal section, they appear as teeth. These are the "cuticular fringes" of Baylis and Daubney (1922).

Angle muscles are absent in the pharynx. The angles of the lumen are attached directly to the tunica propria (Fig. 47). The three sets of surface muscles are each composed of four units, as follows: one anterior median muscle, inserted in the middle of the field, with its origin on the pharyngeal supports of the lip support ring. A second muscle also lies in the median line directly posterior to the first. The origin of this one is in the tunica. It extends to the posterior end of the pharynx. On either side of the two median muscles is a flat muscle, forming flanking units of the median set. These are thicker and heavier anteriorly than posteriorly. They disappear entirely before reaching the posterior end of the pharynx. All muscles but the anterior medians are shown in Fig. 47.

No nuclei of any kind are to be found in the pharyngeal muscles.

The esophagus consists of an anterior cylindrical portion of nearly equal diameter throughout, and a posterior bulb equipped with a corrugated valve and intestinal valve (cardia) (Fig. 3). The bulb is divided into two unequal parts by a deep constriction, giving an "hourglass" effect in some species. The musculature of the bulb (including both parts) and that of the cylindrical esophagus are distinctly different structures, so that the division is not an artificial one.

The lumen presents a different picture in different parts of the esophagus. In the cylindrical portion it is regularly tri-radiate in cross section with a widening at the angles. This widening is characteristic of this section and absent in the bulb. It represents a longitudinal canal, one

at each angle of the lumen (Fig. 18). The central lumen is in communication with the canals along their entire length. In the anterior bulb, as stated, the accessory canals are absent. Here the angles of the lumen are very acute, and a cross section is more nearly perfectly triangular (Fig. 16). This condition is retained into the posterior bulb. At the corrugated valve the entire lumen abruptly widens, so that the walls of the anterior end stand at right angles to the longitudinal axis. Cross sections would here be tangential to the walls, giving the peculiar appearance shown in Fig. 41. The angles of the lumen are more pronouncedly extended than the intermediate regions, for attachment of muscles. The posterior walls of the valve do not narrow so abruptly, but taper gradually to the intestinal valve, just anterior to which the typical tri-radiate appearance is seen in cross section (Text-fig. 1, j).

It is necessary to point out that the so-called corrugated valve neither is extraordinarily corrugated nor is it a valve. It is a chamber, which, by manipulation of special muscles may have its cubic capacity increased or decreased. It represents a simple dilation of the lumen and is not a true valve, since there is no mechanism for prevention of regurgitation. Dilation of the walls would tend to draw materials into it, which would without further manipulation pass into the intestine. The corrugated valve is then of the nature of a suction pump, and the valve to prevent backflow is at the entrance to the intestine (the cardia).

The cuticula of the entire esophagus is equipped with special thickenings on the external surface for the insertion of the muscles. These are especially strongly developed on the surfaces of the corrugated valve.

Three pointed projections in the form of teeth are found at the anterior end of the cylindrical esophagus. These are in the center of each surface field, and are directed forward into the lumen of the pharynx.

A description of the muscles of the cylindrical esophagus is comparatively simple. The angle muscles are attached to the external surfaces of the accessory canals, some of the fibres passing around to the sides. There are two longitudinal rows of fibres to each angle of the lumen. They may be separated from the surface muscles by the slightly deeper staining reaction (Fig. 18). Of the surface muscles there are apparently eighteen rows, six to each field, but the fibres at any one cross section are hardly separable except at their insertions in the thickenings of the cuticular lining. The bundles are more evidently discrete in longitudinal section. These rows extend the entire length of the cylindrical esophagus. The esophageal glands divide the rows into two groups of three each in each muscle field (Fig. 18). Probably the division of the surface muscles into small bundles of fibres has no other significance than as an adaption for more secure insertion. Modifications of the musculature of the anter-

ior bulb and the anterior third of the posterior bulb consist, first, in the insertion of the angle muscles in the acute point of the angles of the lumen, in the absence of the accessory canals; secondly, in resolving the two rows of the angle muscles into one, possibly a result of crowding; and, thirdly, in the union of each of the three rows of surface muscles of each half-field into a single row. Fig. 16 shows the condition of the muscles here.

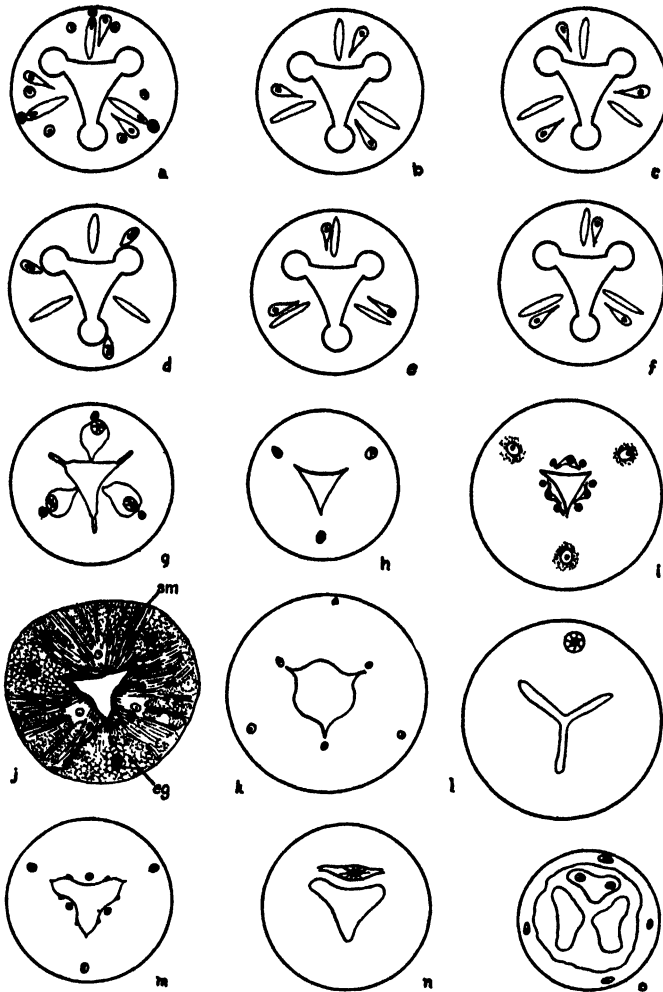
A very complicated series of modifications has taken place in the muscles of the posterior bulb, correlated with the modifications of the lumen into the corrugated valve. In spite of the extensive modifications, all the muscles may be referred to either the angle muscles or the surface muscle type. The bundles of both are divided into anterior and posterior groups, which for the most part have their insertions in the extreme angles or widest part of the surfaces of the corrugated valve. Contraction of the anterior bundles shifts the valve forward and closes the lumen. Muscles of the cardia close the valve into the intestine at the same time. Contraction of the posterior bundles opens the corrugated valve to its widest extent and pulls the entire structure backward, thus producing a suction. The cardia open at the same time, allowing food to enter the intestine. These movements have been observed in living animals. Figs. 40 and 49 show distribution of the muscles.

MUSCLE NUCLEI OF THE ESOPHAGUS.—For convenience the discussion treats the nuclei of the cylindrical esophagus and the bulb separately. The former will be discussed first.

Pertaining to the muscles, there are three types of nuclei, differing in form and staining reaction. The first of these is in the form of the conventional tear-drop (Fig. 39). The acute point is sometimes drawn out into a long thread, which is always directed toward the center of the lumen. Thus the long axis of the nuclei always corresponds to one of the radii of the esophagus as seen in cross section. A single large nucleolus occupies the large end, and sometimes other chromatic elements are present. The length is $19\ \mu$, the breadth $8\ \mu$, the nucleolus being about half the breadth.

The second type of nucleus of the cylindrical esophagus presents no special features. The nuclei are small, oval, or spherical, with a small central nucleolus. The diameter is $6\ \mu$. These nuclei stain rather lightly, and are apt to be overlooked because of their small size (Fig. 42). The third type resembles the tear-drop type, but is more nearly oval, and has an additional odd-shaped nucleolus (Fig. 37).

The distribution of the nuclei is as follows: The first set is at a level between 10 and $40\ \mu$ posterior to the junction of the pharynx and esophagus, i.e., in the extreme anterior end. The nuclei and their positions are



TEXT-FIG. 1.—Diagrams *a* to *f*, inclusive, show muscle nuclei of the cylindrical esophagus. Radially striated cells and those in the gland ducts belong to the sympathetic nervous system. *g*, nerve cells and muscle cells of the anterior bulb. *h*, angle muscle nuclei of the anterior region of the posterior bulb. *i*, esophageal gland nuclei (over the angles), nerve cells (median surface fields); surface muscle nuclei in the middle of the half-fields. *j*, same as *i*, drawn in detail with camera lucida. *k*, third set of muscle nuclei. *l* and *n*, nerve nuclei. *m* and *o*, fourth set and cardial set of muscle nuclei.

illustrated in Text-fig. 1, a. The smallest of the nuclei here (lying in and above the esophageal glands) belong to the sympathetic nervous system. There are six of the small round nuclei (type 2), one in the center of each half-field of the surface muscles. These are not all at exactly the same level, since the figure is a combination of five 10 μ sections. This set completes the list of type 2 nuclei of the cylindrical esophagus. The question of their relation to the muscles is uncertain, except that they belong to the surface muscles, and perhaps originally belonged to the pharyngeal group.

At the same level with these six small nuclei is one set of three tear-drop nuclei (type 1). Before proceeding further, I must point out that no two of these occupy the same radius. This first set of three is located, one in each left half of the surface fields, close to the median esophageal glands, excepting the nucleus in the right submedian field which is closer to the angle of the lumen (Text-fig. 1, a). When I say left half or right half of a field it must be kept in mind that I am looking at each sector as if it were dorsally located, i.e., from the lumenward side. Since the sections are seen from the surface, "right" will correspond to the observer's left.

The second set of tear-drop nuclei is situated just posterior to the nerve ring, and about 0.3 mm. from the anterior end of the cylindrical esophagus (Text-fig. 1, b). These are located in each left half of each surface field, about midway between the angle of the lumen and the gland.

The third set is at a level about 0.38 mm. from the anterior end and thus 0.08 mm. from the preceding set. These are in the middle of the right half sectors (Text-fig. 1, c).

The fourth set is 0.62 mm. from the anterior end and thus 0.24 mm. from the preceding set. They are in the left half sectors very close to the angles of the lumen. These belong to the third type of nucleus, with the extra nucleolus, and probably belong to the angle muscles (Text-fig. 1, d).

The fifth set occurs at 0.88 mm. from the anterior end and 0.26 mm. from the preceding set. Those in the dorsal and left subventral fields are in the right half and very close to the glands. In the right subventral field the nucleus is in the left half and similarly close to the gland (Text-fig. 1, e). They belong to type 1.

The sixth and last set of tear-drop nuclei is at a level about 1.05 mm. from the anterior end and 0.17 mm. from the preceding set. The two in the dorsal and left subventral fields are in the left half and close to the glands; the one in the right subventral field is in the right half and close

to the gland. This set is not far anterior to the end of the cylindrical esophagus (Text-fig. 1, f).

There are 24 nuclei of the cylindrical esophagus referable to the muscles, and all but three of them belong to the surface muscles.

MUSCLE NUCLEI OF THE BULB.—The nuclei referable to the anterior bulb muscles consist of a set of three occupying about the middle of the surface fields. They are located at a level near the anterior end. They are of the small rounded type (type 3) (Text-fig. 1, g).

Two types of nuclei are found in the posterior bulb. One of these is of type 1. The other is similar but much larger, 13 μ in diameter. They possess two nucleoli, one spherical and the other blade-like (type 4) (Fig. 38). Their distribution is as follows:

One set of three (type 4), belonging to the angle muscles, is situated 40 to 50 μ from the anterior end of the posterior bulb, one over each angle of the lumen (Text-fig. 1, h). They are in the unmodified section of the musculature.

The second set (type 1) of six nuclei is situated 80 to 90 μ from the anterior end, one in the middle of each half-field (Text-fig. 1, i). They belong to the anterior surface muscles of the corrugated valve. Other nuclei appearing in the figure belong to the esophageal glands and sympathetic nervous system.

The third set consists of six nuclei lying at the level of the anterior surface of the corrugated valve (Text-fig. 1, k). Three are from the surface muscles and three from the angle muscles. The surface nuclei are located far out from the lumen, and belong to the anterior bundles of surface muscles of the corrugated valve. The angle muscle nuclei belong to the anterior bundles of the corrugated valve. All are of type 1.

The fourth set likewise consists of six nuclei, three of which belong to the posterior angle muscles of the corrugated valve, and three to the posterior surface muscles (Text-fig. 1, m). The surface nuclei are of type 1, the angle nuclei of type 4.

Three more nuclei belong to the muscles of the bulb. These are located in the cardia. Their positions shift somewhat, two or even occasionally three being in one cardium; usually they are distributed one to each cardium. Occasionally one or two may be absent, probably due to degeneration with age. They belong to type 1 (Text-fig. 1, o).

This is a total of 24 muscle nuclei in the bulb, of which 9 belong to the angle muscles; the remainder to the surface fields, considering the nuclei of the cardia as belonging to this category. For the entire esophagus there are 51, of four different types. The significance of these types is a problem awaiting solution. I think that some of the reports of nuclei of the esophageal muscles must be erroneous because of failure to make

proper conclusions as to the nature of the nuclei. At the present time nothing can be done to remedy the situation. The correct conclusions must await a considerable volume of comparative work on different forms.

ESOPHAGEAL GLANDS.—These structures in *Spironoura* do not have the extremely high development that they have in the *Ascarids*. For the greater part of their length they consist of simple tubes (Fig. 18), lacking the complicated branching characteristic of most of the larger parasitic forms. In *Spironoura* the glands extend from the anterior end of the esophagus to the posterior end, the most complicated branching occurring in the posterior bulb.

From the anterior end to a point about 0.3 mm. posterior to the nerve ring, the tubes are practically unbranched in each field. Here the first considerable branching occurs, forming a labyrinth of gland tissue around and between the muscle fibre bundles, and joining the glandular tissue of the three fields around the angles of the lumen. From this point to the end of the cylindrical esophagus the tubes revert to the unbranched condition. In the anterior end of the anterior bulb the second branched area occurs in a similar manner to the first. Through the remainder of the anterior bulb and the anterior end of the posterior bulb the tube again reverts to an unbranched condition. In the interspaces of the modified muscles of the posterior bulb a third network occurs, here with a much greater bulk of glandular tissue. The basic position of the glands changes here also. Each tube, as it enters the posterior bulb, branches or at least widens toward each side, and the lateral widenings from adjacent surface fields join over the angles of the lumen. In the bulb, the bulk of the glandular tissue is found over these angles. The three nuclei of the glands are found in the same relative positions as are the angle muscle nuclei (Text-fig. 1, i and 1, j). The nuclei are found at about the level of the anterior surface of the corrugated valve, or a little farther anterior. The position of the nuclei over the angles of the lumen, instead of over the flat surfaces, has its parallel in *Ascaris* (Mueller, 1931). The nuclei are spherical and have a diameter of about 14 μ . The large nucleolus has a diameter of 6 μ .

The openings of the glands apparently are at the same level for all three glandular tubes. This level is the point of junction of the esophagus with the pharynx. At this point the lumen forms three evident out-pocketings into which the ducts empty. The openings are in the form of narrow slits (Fig. 45).

ARCADE CELLS.—Martini (1926) has given an interesting and minute account of these cells in *Oxyuris robusta*. The fundamental structure of the arcade system is the same in *Spironoura chelydrae* as in *Oxyuris*

robusta, but there are variations apparently of a character quite constant for the several species of *Spironoura* investigated. For the general form of the glands see Fig. 5, drawn from *S. affine*.

There are nine cells in the system, the same number as in *Oxyuris*. The bodies of these nine cells begin anteriorly at about the anterior end of the pharynx. From this point the arcade cells continue backward a variable distance, but always as far as the nerve ring. The anterior ends are thin and duct-like; the bodies of the cells become progressively thicker posterior to the level of their nuclei, about 30 to 100 μ anterior to the nerve ring, the exact positions varying both individually and for the different cells. Posterior to the nuclei the cells become narrower, and in the case of the dorsal cell, bifurcate.

One of the cells lies against the tissue of the dorsal line. Posterior to the nucleus, as stated above, this cell bifurcates; the two branches following the sides of the dorsal line are wedged in between it and the adjacent muscle cells on each side, one right and one left. Two cells lie over each dorso-lateral angle of the lumen of the esophagus. The ventral one of each of these pairs, while attached anteriorly in this position, bends diagonally ventralward across the lateral line and continues backward at the lower edge of the latter. It is at this point that the nucleus is found. There are two cells over the middle of the ventral muscle fields, one on each side. These are close to the ventral ones of the two pairs just described. The last two cells lie one on each side of the ventral line. The positions of all the cells (at their nuclei) may be seen in Fig. 18. It is clear that this arrangement is essentially the same as that described by Martini (1926) for *Oxyuris*.

As a matter of convenience, I have described the cells as discrete units. No one of them is, however, independent of the others. A network of cytoplasm interconnects all nine, beginning anterior to their nuclei and continuing nearly to the nerve ring. In *Spironoura* the arcade system may be considered as a glandular syncytium, forming a more or less complete cylindrical network around the esophagus.

The cytoplasm of the entire syncytium has a very marked staining reaction. It is filled with large spherical granules. In ordinary Delafield-eosin staining of sections, the granules take an intense black, and they are very much overstained when other tissues are about correct. The granules take nuclear stains much more deeply than the nuclei themselves. In toto mounts the best stain is borax-carmin. No stain at all also gives good results, since they are naturally pigmented.

I cannot consider these as amphid glands, although the anterior extremities approach very close to the base of the amphids.

INTESTINE.—This is the portion of the digestive tract often spoken of

as the "chyle" intestine, and presumably is that portion of the tube in which the actual digestion takes place. In *Spironoura* the food is probably predigested by the host, so that the function is more absorptive than digestive. The tube is shaped somewhat like a slender baseball bat, with the large end directed forward, and it makes contact with the esophagus in a circular collar (Figs. 3 and 40). It would be useless to give measurements of the diameter of the intestine, since it varies greatly, depending somewhat on the amount of material contained within it, and possibly on factors of contraction. The large end (anterior) may completely fill the entire body cavity and have the posterior bulb partly telescoped into it, causing the walls at the end to be turned in and backward, or it may be no greater in diameter than the bulb and not at all telescoped. The point is mentioned because of the tendency to use measurements of the diameter of the intestine as a specific character.

The shape of the lumen in cross section differs at various levels of the intestine. Anteriorly it is circular, the epithelial cells flattened, and there are no projections into the lumen. At about the level of the junction of the anterior and middle thirds of the tube, the cells of the dorsal and ventral sides of the epithelial lining elongate and become columnar in such a manner as to form a dorsal and ventral projecting ridge corresponding somewhat in shape to the typhlosole in annelids. The ridges reach their maximum thickness at about the middle of the intestine, and here a cross section shows a lumen somewhat in the shape of a letter "N" (Fig. 15). The ridges gradually disappear posteriorly. Just anterior to the rectal sphincter, all the epithelial cells become columnar, and the diameter of the lumen becomes so constricted as to be completely eliminated. This plug of cells is in the nature of a valve into the procotodaeum (Fig. 51).

Earlier workers have described the hyaline inner border of the intestinal epithelium as cuticular (Looss, 1905), or as a material accrued from the intestinal contents. Later workers (Hetherington, 1923, and others quoted by him; Mueller, 1929) consider this border to be made up of immobile cilia. In *S. chelydrae* the border is very plain, wide, and distinct, especially in the anterior region. In favorably stained material the transverse striae are clear, and very much suggestive of cilia. (Fig. 50). The distal ends of the cells also frequently show fibres, so as to suggest a neuromotor apparatus, a condition noted by Mueller in *Ascaris*. No basal granulae were seen, but a thin dark line at the inner borders of the cells may be these structures.

On the other hand, the border does not continue throughout the length of the intestine, being absent posteriorly. If the borders really represent cilia, they must be in a very degenerate condition in *Spironoura*.

Seen in toto mounts, surface view, the epithelial cells are hexagonal to irregular in shape (Fig. 48). Anteriorly the cell boundaries are distinct, but become less and less so posteriorly, depending somewhat on the staining medium. At about the region of the intestinal sphincter the cell boundaries again become distinct.

The shape of the cells varies considerably according to the amount of fecal material contained in the lumen. All forms from flattened to columnar may be found, and the individual cells vary within limits in this respect.

The nuclei are oval to round, and contain two nucleoli, one of which stains deeply in basic stains, and the other very lightly in the same medium. The position of the nuclei is central or slightly eccentric, near the lumen in columnar cells. The cytoplasm is vacuolar or fibrous according to the stain, and Mallory's triple probably gives the best results (Fig. 48).

As described by Looss (1905) for *Ancylostoma*, the rectal sphincter has but one nucleus (Fig. 51). The sphincter lies against the rectal ligament posteriorly, in a circular depression around the posterior intestinal plug or valve. Contraction of the sphincter serves to cut off the lumen of the intestine from that of the proctodeum, which could not be accomplished without the aid of the columnar cells of the epithelium. It is possible that the columnar cells are more or less contractile of their own power.

The posterior end of the intestine projects more or less into the proctodeum, posterior to the sphincter. This portion serves as the basis for the connection to the proctodeum, by means of the intestino-rectal ligament to be described later.

Looss (1905), when studying the European hookworm, first described the anastomosis of fibres around the posterior end of the intestine, and commented on what he considered as the probable function of the organ. Except for minor details, his description might have been written for *Spironoura*, so exactly do the two structures coincide. Looss considered the fibres to be muscles and called them the intestinal muscles.

Just anterior to the rectal sphincter (30 to 40 μ) are found two small nuclei, one on each lateral surface of the intestine and closely applied to the outer wall, that is, to the tunica. A small cytoplasmic body surrounds each nucleus, and radiating out from the central body are numerous fine strands of tissue. These also are applied closely to the tunica. Some run posteriorly and end in or at the anal sphincter, others branch around the intestine dorsally and ventrally, and still others project forward for a distance, in some specimens, of 2 to 3 mm. All of the strands anastomose freely among themselves, and the strands from one

cell anastomose with those from the other. The result is a continuous web of fibres encasing the end of the intestine. In addition, many fibres run across the intervening space of the body cavity to the lateral and median lines, to the ovary or testis, or to the surfaces of the muscle fields. Those around the intestine appear to be encased in a clear hyaline substance, possibly part of the cytoplasm of the two cells, or a secretion of them. The appearance of the muscles in cross section is shown in Fig. 43. This section is cut between the nuclei of the cells and the intestinal sphincter. Since most of the strands in this region are longitudinal the appearance is of discrete longitudinal muscles.

Looss considers these muscles as functioning in forcing fecal matter from the intestine into the proctodeum. An additional function would be the reinforcement of this region of the tract to prevent rupture through too great distention by accumulated contents.

The proctodeum is formed from an invagination of the external cuticula. The invagination becomes covered by a series of external ligament cells, which serve also for support, being attached in various ways to the body wall. The transverse diameter is greater than the dorso-ventral diameter, and the anterior end is considerably wider in both dimensions than the posterior end, which narrows to a transverse slit at the anus. The general shape may be seen in Figs. 3 and 19.

The lining is composed of two layers of cuticula. The outer layer is in contact with the ligamentous cells. The inner layer is very thin, little more than a membrane, and is connected only loosely to the outer layer by a reticulum of fine fibres. These fibres are long enough, dorsally and ventrally, to reach approximately half-way across the lumen of the rectum. When the rectum is empty these fibres are extended, and the inner layer of cuticula suspended thus from the dorsum is in contact with that attached to the venter. In this manner the rectal lumen may be completely occluded (Fig. 19).

The entire cuticular wall of the proctodeum is covered externally with a layer of cells (called "ligament" cells by Looss). These cells are in contact with the cuticular wall of the proctodeum and conform to the shape of the outer wall. In addition, certain cells send out processes laterally to form a supportive bridge and dorsally to form a thick mesentery. The boundaries of the cells are sometimes difficult to distinguish, and the nuclei therefore are used to ascertain the cell number, which is apparently ten.

In the female, three of these cells form the intestino-dorsal region of the proctodeum. A thick median process in the sagittal plane projects to the dorsal line, and is firmly secured to it in such a manner as to suggest a thick mesentery (Fig. 19). Each latero-anterior surface is

covered by a large cell, the two meeting ventrally. Lateral projections of these are fused to the lateral lines to form a complete transverse supportive bridge (Fig. 19). The ends of these cells are usually free in nematodes.

The entire proctodeum of the male is a cloaca, since the entrance of the genital canal is almost at the extreme anterior end, and the canal is thus a common duct throughout its length.

As in the female, the anterior end of the rectum is of considerably greater diameter than the posterior end and is dorso-ventrally flattened. The lining cuticula is the same as in the female.

The arrangement of the covering and supportive cells is also the same as in the female, with the following exceptions: (1) There are two cells in the dorsal support (mesentery) instead of one (Fig. 10). (2) The bases of the two cells forming the genital ligament (Fig. 10), and a cell lying between the preanal grooves must be added to the list of ventral cells. This adds four cells to the total, making 14 cells in all.

The genital ligament consists of two cells lying one on each side of the end of the genital tube (Fig. 27). Their posterior ends cover the antero-ventral end of the cloaca. Thus there are five cells in the intestino-proctodeal ligament of the male.

Directly dorsal to the anal opening, and thus at the extreme posterior end of the dorsal wall of the cloaca, an evagination of the cuticula into the body cavity occurs, forming a pocket behind the gubernaculum (Fig. 9). The lumen of this pocket is closed when the spicules are extruded, and slightly open when they are withdrawn. Just anterior to the evagination forming this pocket, a second evagination occurs which is the true spicular canal (Fig. 9). The inner end is open, and the walls lose their cuticular appearance and merge imperceptibly into the gubernaculum posteriorly and into the covering cells of the cloaca anteriorly and laterally. The length of the canal is so negligible as to make the structure little more than a cuticular collar around the ends of the spicules.

The gubernaculum is of a hard, non-pliable material, quite different from the cuticula of the body wall or cloaca. It is, from staining reaction, of the same material as the spicules. In Mallory's triple, both gubernaculum and spicules stain a bright red, while body wall and cloacal cuticula are a light blue. The contrast is quite striking.

In form, the gubernaculum is an oblong flat plate, flaring somewhat at the inner end. The anterior surface is gently concave both laterally and longitudinally; the posterior surface is correspondingly convex. Across the anterior surface above the middle there is a transverse ridge. A corresponding groove across the dorsal surface of the spicules fits

against this ridge, and when the gubernacular muscles close the spicular canal at the bottom, the spicules are tightly held in place by this ridge. On the posterior surface of the gubernaculum is a pointed projection arising near the lower end and curving away from the gubernaculum, much like the barb of a fish hook. This barb is tightly socketed in the anterior wall of the post-gubernacular pocket (Figs. 9 and 10).

The spicules are discussed here because of their intimate structural relation to the cloaca. Their lengths vary from 3.2 mm. to nearly 4.0 mm. When not extruded, the anterior ends lie in the region of the pseudo-sucker, thus being about one-fourth the total body length.

At the anterior end of each spicule is an oval mass of four cells. These lie in the groove formed by the open end on the ventral side. The muscles of the spicule sheath are inserted in the mass of cells posteriorly, and the two retractor muscles have their insertions anteriorly.

The form of the spicule may be seen by observing the cross section (Fig. 46). This section is representative of the structure for most of the length, the two ends, however, varying. The body of the spicule is hollow, and inside the tube is a fibrous tissue. There are two alae, attached laterally, with their edges directed ventrally. The proximal edges of the alae are also hollow. Anteriorly the canals of the alae join the central canal by breaking through the walls. Anterior to this point a cross section resembles a letter "D" with the flat surface on the ventral side. Shortly anterior to this point a portion of the ventral wall disappears. Here the fibrous material inside the canal joins a similar material outside the opening but enveloped in the sheath, and here two nuclei are constantly present (Fig. 44). These perhaps are gland nuclei, and the material inside the tube represents a duct or secretion. The opening in the ventral side of the spicule widens anteriorly until, at the point where the retractor muscles begin, the spicule disappears.

Close to the posterior end of the spicules the lateral alae likewise disappear, leaving only the central body part to form the point. The canals seem to be open at the ends so that any secretion contained within them could be passed to the outside at the posterior tip.

Two retractor muscles of the spicules run forward from the end of each spicule to a point about 2.5 mm. in front of the anterior end of the spicule, where they are inserted in the body wall just dorsal to the lateral line. Each muscle has a single nucleus.

The protractors of the spicules are considerably more complicated. They are attached, as already mentioned, to the anterior spicular cell mass, and also to the rim of the opening in the end of the spicules. They form a continuous sheath around the spicule to the end of the guber-

naculum, where they separate into several bundles, with attachments in the body wall posterior to the gubernaculum on the ventral side, in the lateral body wall, and in the dorsal body wall.

Each branch has a separate nucleus. Since there are four branches, each spicule sheath must be considered as a syncytium of four cells.

FEMALE REPRODUCTIVE SYSTEM

As with the majority of nematodes, the ovaries are double in *Spironoura*; if the condition in free-living forms may be taken as primitive, *Spironoura* is representative of a condition near that of the primitive nematode. This is not to be considered as meaning a simple condition. Beyond modifications designed to facilitate quantity production, the primitive condition is apt to be more complex than that found in parasitic forms of long standing. Quantity production of embryos or eggs is often associated with loss or atrophy of certain organs. Thus in *Dracunculus*, there is a complete loss of all muscular organs of the uterus, glandular epithelium, and all the vagina, in late adult stages.

GENERAL DESCRIPTION.—The length of each ovary in *Spironoura chelydrae* (counting uterus, glands, etc., but not vagina) is roughly twice the entire body length. The vagina is 3.5 to 4.5 mm., or somewhat less than one-third the total body length. This length is extraordinary when compared with other species of the genus. In *S. catesbiana* the vagina is less than 0.5 mm. long, and in *S. affine* it may reach a length of 1.25 mm., which two figures represent the minimum and maximum of all other North American species.

While the length of the tubes is in no way comparable to forms like *Ascaris*, it is nevertheless great enough to make it very difficult to follow the tubes through in toto mounts of the entire worm and to determine the exact disposition of the organs located. Longitudinal sections afford the best studies, not only of gross structure but also of histological details. I have had recourse also to dissections, and in favorable instances, I was able to remove and disentangle the entire genital system.

The extremity of the anterior ovary (Fig. 56) lies a short distance posterior to the posterior bulb of the esophagus (a). Figs. 56 and 53 show the gross morphology of both the tubes. The coils here are more or less constant for all species of the genus which I have studied, the greatest variation resulting from the shorter vagina in the other species.

HISTOLOGY OF THE FEMALE SYSTEM.—The extremity of each ovary is capped by an apical cell (Fig. 29). Around it is a thin membrane, which also covers the entire ovary, becoming modified in the more proximal sections. This membrane is nucleated (Fig. 28), apparently is a

syncytium in the distal part, and is evidently a true epithelium. It is exceedingly thin at the free (distal) extremity, not exceeding $1\ \mu$ in thickness. It thickens proximally, and the cells become spindle-shaped, the long axis of the spindle coinciding with the long axis of the tube. The bodies of the cells project inwardly into the lumen of the ovary, giving in cross section the appearance of longitudinal muscle fibres lying along the inner surface of the ovarian membrane (Fig. 28).

Some 20 to $30\ \mu$ from the apical cell the true ovarian epithelium may be seen, composed of columnar cells projecting at an angle into the lumen of the tube. The primordial germ cells, which may be seen in the tube at this point and farther along, are produced from the epithelium.

The gametes are characteristically formed in various parts of the tube of the ovary. Those nearest the apical cell, recognizable as germ cells, are oval or round, about $4\ \mu$ in diameter (greatest), with a distinct large nucleus. Next is a region of mitotic activity, which produces oögonia that become progressively smaller toward the proximal end of the tube. The reduction in size takes place largely in the cytoplasm, while the nuclei become rather irregular in shape (Fig. 31). The reduction in size continues for only a short distance.

Up to this point in the tube (about 0.5 mm. from the end) no rachis is visible. Between this point and a point 0.3 mm. farther along, the cells become visibly larger; this increase in size continues to the end of the ovary, where the cells have reached their maximum bulk, not counting the shell material. At about the region where the growth in the oöcytes begins, a distinct rachis becomes visible. It is formed from strands left trailing behind the gametes as they move along the tube. Even before the central rachis is visible as such, isolated strands may be observed among the gametes. Soon after the rachis is formed the gametes become radially arranged around it, each cell assuming a more or less triangular shape, with the acute angle toward the rachis (Fig. 26). Here it might be noted that what appears in cross section to be a regular radial pattern, is in reality spiral in form, as may be seen from study of longitudinal sections and toto mounts. The edges of the cells overlap, so that the cell boundaries in cross section become more and more difficult to see. In the more proximal regions of the rachis tube this overlapping gives the appearance of a syncytium.

At first the rachis is straight, following the center of the tube. Farther down, where the cells have become large enough to fill more than half the tube, it begins to zigzag from side to side (Fig. 53). The overlapping gradually becomes more pronounced, and the final result is that the cells become large enough to fill the entire width of the tube, and proceed henceforward in single file. The most pronounced pressure is

now in the direction of the long axis of the ovary; hence the cells assume the form of circular flat plates (Figs. 21 and 28). In fully mature females the rate of production of eggs becomes so great that the cells become flattened to such an extent that the nuclei may bulge out on both sides.

The portion of the tube not productive of oögonia is technically oviduct, although for reasons of convenience I have made no distinction, there being no definite separating point. At the end of the oviduct is a short constricted region, the function of which is evident. Although not much longer than an egg (about 0.2 mm.), the gametes pass into it in the form of a flat plate and emerge in the typical oval "egg" shape, which is retained henceforward. A columnar epithelium lines the inside of the constriction, replacing the spindle cells of the oviduct. The cells are here angled sharply toward the proximal end of the genital tube, and probably serve somewhat as a valve to prevent movement backward, and partly as a mold for the eggs. Since the structure of the cells resembles that of the cells of the shell gland, it will not be described here.

It is necessary to point out that the designation of the next part of the tube as shell gland is subject to question. Looss (1905) considered a similar organ in *Ancylostoma* as more in the nature of a vitellarian gland, and considered the uterus as the functional shell gland. There is considerable evidence to support his conclusions. On the other hand, I have studied the cytoplasm of the cells entering the gland and those leaving it, and can detect no difference which would indicate an added food supply. Certainly there is no added bulk. There is a refractive, mucus-like layer around the eggs as they emerge from the gland. It is my opinion that this substance during subsequent passage through the uterus hardens into a shell. In the semi-liquid state, while in the inner end of the uterus, it would not prevent passage of the sperm in fertilization. I think it altogether probable that the uterus elaborates some substance that serves to harden the shell.

The shell gland is a relatively short portion of the tube. The diameter depends on the activity, presence of eggs, etc., but it is usually rather narrower than the oviduct. The outer epithelium presents no special modifications. The lining is composed of a series of epithelial cells of columnar form, the cytoplasm of which is filled with numerous large spherical vacuoles. The basal cytoplasm is finely granular, and the boundaries of the cells indistinct. No evident cell boundaries are to be seen in the lumenward ends, and discrete strands of cytoplasm may often be seen in the lumen (Fig. 23). The nuclei are small and situated near the base of the cells.

Spermatozoa are most numerous in the extreme inner end of the

uterus near the junction with the shell gland. This region is in no way different in structure from the remainder of the uterus, but it seems advantageous to designate it as "seminal receptacle" simply to call attention to the sperm present. Penetration of the eggs by the sperm takes place here. The wall of the uterus has a totally different arrangement from any described portion of the tube. Around the outside is an arrangement of circular contractile cells; the lining is composed of a low epithelium, which is, however, structurally quite different from the epithelium of the shell gland. It also has a different staining capacity.

The outer muscular layer is composed of spindle-shaped cells, broad and flat on the inner side applied to the epithelium and with quite apparent longitudinal (transverse to the longitudinal axis of the tube) ridges running most of their length around the outside. The appearance of these ridges, which contain the nuclei of the cells, when seen in toto mounts, suggests discrete circular bands (Fig. 56), and the bases of the cells appear to be a middle layer. Sections (especially longitudinal) show the true relation of the parts. At the inner end of the uterus one muscle cell is sufficient to reach most of the way around the tube, especially when in a state of contraction; this increases the impression of complete bands. At the end adjoining the vagina the cells are much shorter, while the diameter of the tube is larger, so that as many as three or four cells are necessary to complete the circumference. This is not to be taken to mean that the ends of the cells abut each other in a truncate manner. The cells are spindles, more or less sharply pointed at each end, and overlap the ends of the cells on each side. The arrangement, therefore, is more spiral than circular. Cell boundaries are very indistinct in cross sections (Fig. 35) and toto mounts, but show plainly in longitudinal sections.

The epithelium consists of oblong flattened cells, in the middle of each of which is an irregular cytoplasmic lobe into the lumen. The longitudinal axis of these cells corresponds to the longitudinal axis of the tube; therefore, it is at right angles to the outer layer of muscle cells. The inner cytoplasmic lobes contain the nuclei (Fig. 35). A large quantity of mucous material is elaborated by this epithelium, the function of which is, partly at least, to lubricate the passage of the eggs. Quantities of the substance are passed on into the vagina with the eggs.

The appearance of the cytoplasm is quite different from that of the shell gland epithelium. The numerous vacuoles are absent, and the cytoplasm is dense and homogeneous.

VAGINA.—This portion of the genital tube differs from the uterus in two quite apparent ways. First, the epithelial lining is absent, and its place is taken by a cuticular lining; second, the spiral of muscle cells

around the outside have their surfaces reversed, that is, the contractile lamellae are outside, at least the heavier portion, and the cytoplasmic cell bodies containing the nuclei are inside and completely surrounded by contractile substance (Fig. 2). In general arrangement, the muscle cells resemble those of the uterus. They are spindles encircling the outside of the tube, with the ends of the cells overlapping; the arrangement is, therefore, spiral and has much the same appearance as the uterus, except that the muscle bands never appear to be separated. They are evidently much more powerful in contractions than the uterine muscles. The muscles are attached loosely to the inner cuticular lining by means of radially arranged connective tissue fibres (Fig. 30). The same fibres attach the cells to each other laterally, allowing independent movement, which is a necessity when the cells are spirally arranged. Only two nuclei occupy the fibres, both near the vulva (Fig. 2).

Near the inner end of the vagina the lumen is regularly circular in cross section. Toward the vulva, however, in the absence of eggs, the lumen has a regular cross or diamond shape (Fig. 30). The cuticula is composed of eight longitudinal plates, joined to each other laterally; when an egg is passing through, the plates have the appearance of a continuous circular piece; when the tube contains no eggs, the lumen is constricted and the cuticula bends inward at each alternate joint, while the others bend outward. Because of the intervening connective tissue fibres, it is not necessary for the muscle cells to conform to the shape of the cuticular lining.

At the junction of the vagina with the body wall at the vulva, there is a modification of the ventral line to form a peculiar pad-like organ surrounding the end of the vagina. This may possibly be, as sometimes described, an organ glandular in nature. The arrangement of the cells is shown in Fig. 2. I incline to consider it a purely mechanical buffer. A special vulvar ganglion lies just posterior and to one side of the pad, which is usually called the "vaginal gland." That the pad has some special significance is evidenced by the exceptional number of large nuclei in it—about four on each side and behind, and at least a dozen anterior to the vulva. The pad is separated from the ventral line tissue, and the nuclei are of different form. No ducts of any kind could be found.

Between the end of the spiral muscles of the vagina and the vaginal gland there is a very large sphincter muscle surrounding the cuticular lining. This is a single-celled organ (Fig. 2).

MALE REPRODUCTIVE SYSTEM

GENITAL TUBE.—Like the ovary in the female, the testis of the male begins with a single apical cell (Fig. 32). This extremity lies posterior to the ventral sucker, the exact position depending somewhat on the state of maturity of the individual. From the apical cell the testis runs forward to a level a short distance posterior to the posterior esophageal bulb (Fig. 12), whence the tube turns backward. About the middle of the body the testis ends. It is followed by a large sac-like seminal vesical continuous posteriorly with the seminal gland. The latter is nearly a third as long as the body, the posterior extremity lying about midway between the pseudo-sucker and the cloaca. Here the tube is again constricted, and at the posterior end of the constriction is a valve, opening into a peculiar muscular area which I am calling the ejaculatory duct. The latter is divided into two evidently different portions, the proximal portion being glandular as well as muscular. The posterior extremity of the ejaculatory duct joins the cloaca ventrally near the anterior end, and it is held in place by the genital ligament.

The male genital tube is thus divided into five quite different portions: (1) testis, (2) seminal vesicle, (3) seminal gland, (4) valve, and (5) ejaculatory duct, which latter is itself subdivided. If the proximal portion of the testis be considered as vas deferens, then there are six morphologically different parts of the tube. This is evidently a greater number than has been recognized for the majority of nematode species. Note that in this species there is no organ comparable to the ejaculatory duct of some species in which the walls are enveloped by *circular* muscles.

TESTIS.—Anatomically the testis corresponds very closely to the ovary of the female. The extremities of the two tubes are almost identical, though some differences are noticeable at the proximal end in contact with the seminal vesicle. The apical cell at the distal extremity of the testis is possibly somewhat smaller in proportion than the apical cell of the ovary (Fig. 32). The outer epithelium at the extremity is composed of an exceedingly thin cellular layer, and lined with a low germinal epithelium. This latter is usually indistinguishable from the germ cells within the lumen, so tightly is the entire mass packed into the tube.

The outer epithelium gradually changes in character from the distal to the proximal end of the testis. Beginning as a thin membrane, the cells become more and more elongate and spindle-shaped, their long axes corresponding almost to the longitudinal axis of the tube. These spindle cells never become absolutely longitudinal, but are arranged in long spiral rows around the testis. Tangential longitudinal sections are necessary

to show this peculiarity. Such sections also show the contractile fibres to best advantage. As in the ovaries, the cytoplasmic non-contractile bodies of the cells project into the lumen (Fig. 34).

A short distance from the apical cell, the germinal epithelium disappears as a definite lining layer. At about the point of disappearance the spermatogonia begin to show a definite increase in size, which is progressive to the end of the testis. The cells form around a rachis, attaining as in the ovary a definite radial disposition (Fig. 34). This arrangement is retained to the end of the testis, and the spermatocytes never attain a size comparable to that of the oöcytes.

SEMINAL VESICLE.—This portion of the tube is generally spoken of as a storage region (Fig. 12), but that is not its principal function. Here occur the peculiar reducing phenomena common among nematodes. Temporary storage may take place, but if it does, it is probably in the ejaculatory duct.

The walls of the seminal vesicle consist of only a simple flattened epithelium. There is no lining epithelium.

SEMINAL GLAND.—I have hesitated to call this gland a cement gland. There is no evidence that its secretion is used to seal the male to the female during copulation as in some nematodes. In the absence of any real knowledge concerning its function, I prefer the noncommittal term *seminal gland*. The epithelium lining this part of the tube is evidently productive of a large amount of mucous material, but whether or not it is nutritive is an unsettled question.

The structure of the seminal gland is almost an exact equivalent of the cement gland of *Ancylostoma* as described by Looss (1905). The epithelium is columnar and very highly vacuolated, and the cells incline in the tube toward the proximal end. The lumen is usually obliterated by the ends of the cells. In general the structure is very much like the shell gland of the female. The secretion stains a light blue with Mallory's triple, both in the lumen and in the vacuoles. As in the shell gland of the female the best studies are afforded by longitudinal sections (Fig. 25). All the nuclei are crowded near to the basal region of the cells, in some cases being in contact with the thin outer epithelium, which does not differ from that of the seminal vesicle.

Between the seminal gland and the labyrinthine tube of the ductus ejaculatorius, there is a narrowly constricted region. The proximal end of this region contains fibrous epithelial cells of a columnar shape, arranged so that their lumenward ends extend into the labyrinthine tube. This forms a sort of a one-way valve evidently designed to prevent backward movement of the sperm in the tube. The arrangement of the cells is shown in Fig. 24.

DUCTUS EJACULATORIUS.—The valve described above opens directly into a portion of the genital tube with a very interesting structure. The entire canal consists of a reticulum of muscular fibres anastomosing in every direction (Fig. 24). The spermatozoa pass through the spaces in the reticulum; thus they must here take a very irregular course. Nuclei are scattered here and there through the fibres of the reticulum, but are usually found near the periphery. The entire tissue is a complicated syncytium. Near the middle of the ductus ejaculatorius, glandular epithelial cells replace most of the contractile fibres, and become more numerous toward the proximal end. The nature of their secretion is also problematical. With iron hematoxylin or mordanted Delafield's hematoxylin the vacuoles and droplets in the reticular spaces stain a deep blue or black (Fig. 36). Lacking the hematoxylin, they will stain with eosin.

At the end of the ejaculatory duct is a very short tube composed of flattened epithelium. This region is entirely encased by two large ligamentous cells, one on each side and joining each other dorsally and ventrally. The posterior ends of these two cells are continuous with the ligamentous cells suspending and covering the cloaca. Their function is to form a connection between the genital tube and the lumen of the cloaca. These cells I am calling the *genital ligament* (Fig. 27).

The accessory reproductive organs of the male (special muscles, spicules, gubernaculum, and posterior papillae) are discussed in connection with the systems with which they are associated.

MUSCULATURE

As in all oxyuroids, the musculature is meromyarian and platymyarian. The total number of somatic cells is 132. These are divided equally among the four fields, thus 33 in each. Each field has 16 cells in the row adjacent to the median lines, and 17 in the row adjacent to the lateral line. Details of their form and arrangement differ from the ordinary condition as described for such forms as *Ancylostoma*, *Ascaris*, and *Oxyuris*, or for any other with which I am acquainted. The differences will be noted as described.

At the anterior extremity two cells from each field have their insertions in the thickenings of the ring support of the lips, that is, in the angle supports and the pharyngeal supports. These eight cells (designated as half-cells by Martini) are truncate at their anterior ends and are markedly shorter than the following cells.

The second set of eight cells have their anterior ends near the posterior end of the pharynx. From here to the level of the esophageal bulb, four cells may always be seen in cross section in each muscle field.

More, of course, may be seen if the cross section is taken near the ends of the cells where overlapping occurs. Fibres from the second set of eight cells form the septum muscularis.

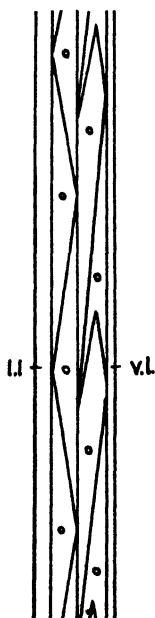
The somatic muscle cells have two quite different forms. This is a modification I have not met with elsewhere. Alternate cells in each field, in the rows adjacent to the ventral and dorsal lines, are bifurcate at the posterior extremity and single pointed anteriorly (Text-fig. 2). All the remainder of the cells are spindles, but not in any case "rhomboids" as described by Martini (1916) for *Oxyuris*. The bifurcate cells have the anterior point at the median lines, and one of the posterior points at the ventral (or dorsal) line and one at the submedian line. The cell of the same row just behind it has the anterior point in the split end of the bifurcate cell anterior to it and the posterior point at the ventral line, wedged in between the ventral line and the anterior point of the next posterior bifurcate cell.

The cells of the rows adjacent to the lateral lines on each side have also a unique arrangement. Both ends of each cell are adjacent to either the submedian line or to the lateral line, alternating in this respect throughout the length of the row. All these cells are therefore triangular, with a very long base against a submedian or lateral line and with the other two sides equal and much shorter.

It is clear that none of these cells could be described as rhomboids although the rather indefinite term "spindle" might fit any but the bifurcate type.

Mueller (1929) described some inclusions within the cytoplasm near the extremities of the muscle cells in *Ascaris*. In some of the cells in *Spironoura*, in a similar position, a single large body nearly as large as the nucleolus, may frequently be seen. Most of these are to be found in the anterior body region. They stain very deeply by Heidenhain's method.

In addition to the bodies apparently confined to the extremities of the cells, any number of other cytoplasmic inclusions of various sizes and position may be found in any of the cells (Fig. 54). These apparently will not stain without mordanting with iron alum. Using this mordant with



TEXT-FIG. 2.—Schema of the somatic musculature. l.l., lateral line; v.l., ventral line.

Delafield's hematoxylin, and countering with eosin, one may stain these inclusions black, the general cytoplasm a purplish blue, and the contractile lamellae red.

The contractile lamellae in cross section appear as a letter "U" (Fig. 59), with the open end turned toward the hypoderm. The ends of the arms of the "U" may be bifurcate also.

The anal muscles are referable to the "H" type as described by Martini (1916) for *Oxyuris*, i.e., a double vertical row inserted dorsally between the dorsal edge of the lateral lines, and ventrally on either side of the ventral line posterior to the anus and in the dorsal side of the rectum. The two rows are connected by a transverse cytoplasmic bridge containing a nucleus. The system is much more highly developed in the male than in the female. The entire series of strands is referable to a single cell in the female; to several cells in the male.

ACCESSORY MUSCLES OF THE MALE.—The pseudo-sucker of the male lies just anterior to the ventral oblique muscles and doubtless represents a modification of these. It probably does not represent a true sucker, yet it seems likely that it is a functioning organ. I find it difficult to consider the structure as a degenerate organ; I think it much more likely that it represents an organ in process of development (phylogenetically) by means of concentration of fibres.

The muscles of the pseudo-sucker consist of 22 or 24 pairs of fibres, each single strand representing a single cell, the ventral ends of which are inserted in or near the midventral line. The dorsal insertions are in the lateral body wall on either side between the ventral edge of the lateral line and the dorsal edge of the subventral longitudinal muscle fields (Fig. 58). The ventral insertions are grouped close together, the dorsal ends considerably farther apart, so that the whole group of muscles on one side has the general shape of a fan. The longitudinal muscle bands of the subventral fields pass under the pseudo-sucker muscles, that is, between them and the body wall.

The ventral insertions are arranged in such manner that they form an oblong oval, the long axis coinciding with that of the long axis of the body. The ventral line lifts up from the body wall and passes over the base of the insertions and between the strands of either side.

Each separate muscle strand contains a nucleus, which occurs about two-thirds of the distance from the ventral end. They do not lie in the contractile fibres but in a bulge of non-contractile cytoplasm on the inner surface. This cytoplasm is granular and alveolar, and usually contains several small bodies which stain very much like the nucleolus and appear like small nuclei. These cells are distinctly like the somatic muscle cells

in general arrangement. At the insertions each muscle may break up into several strands which have the appearance of a frayed end of a rope.

The ventral oblique muscles are in all respects like the pseudo-sucker muscles, except that they are all inserted exactly in the midventral line. The fibres from the two sides mix into a network at the body wall, and the cells are parallel (Fig. 58). The muscles begin at the anterior extremity of the preanal grooves and end at the posterior edge of the pseudo-sucker. The fibres of the anterior end of each muscle are attached obliquely forward from the ventral insertions. The few anterior muscles cross the posterior ends of the pseudo-sucker muscles. The ventral obliques number from 42 to 44 pairs.

ACCESSORY SOMATIC MUSCULATURE OF THE MALE.—Because the male has a set of muscles not present in the female, it is necessary to discuss a part of the somatic system as distinct from the female system. All somatic elements of the female are present in the male also, but in addition the male has a field of cells lying between the ventral edge of the lateral lines on each side and the dorsal row of ordinary somatic muscle cells of the subventral fields (Fig. 58). I call this additional field of cells the accessory somatic muscles of the male. Seurat (1918) mentions this system of muscles. The cells of this accessory field are only about one-sixth as broad as the ordinary somatic muscle cells, but are longer proportionately, being about half the length of the ordinary somatics. At the widest part (just anterior to the cloaca) the accessory fields are about six cells wide, and the individual cells are here somewhat wider than those at the anterior extremity. The bands end anteriorly at about the level of the esophageal bulb, and posteriorly near the anus.

The course of the individual cells is somewhat variable. Anterior to the pseudo-sucker, the anterior ends are either at the edge of the lateral lines or at the edge of the ordinary somatic muscle fields. The posterior ends are at about the middle of the bands. At the level of the pseudo-sucker they become more regular in their arrangement. The anterior ends are at the dorsal edge of the ordinary somatic muscle field. The posterior ends are at the edge of the ventral border of the lateral lines; thus their course is diagonal to the long axis of the body. The effect of this arrangement probably is to counter the ventral oblique bands which cross the accessory bands at an acute angle.

EXCRETORY SYSTEM

In general organization the excretory system in *Spironoura* belongs to the "X" type as described by Martini (1916) for *Oxyuris curvula*. In details it varies from any of the described systems. The excretory bridge

is regularly composed of three cells forming a syncytial longitudinal bar, beneath which the four canals meet at the excretory pore. The space beneath the bar appears in toto mounts like a large bladder.

The interior of the excretory canals usually is filled with a finely granular material which stains with almost any ordinary cytoplasmic stain. In toto mounts the material may be seen to be concentrated at odd points along the tubes, sometimes in sufficient quantity to cause slight distention.

Studies on the excretory canals in living specimens have yielded some interesting points of information. Slightly immature specimens were used, since they are more transparent. Specimens were placed on a slide in normal saline solution or water, wedged in between two cover slips, and covered with a third slip. This prevents excessive motion, and many details of internal anatomy are clearly visible. The excretory canals may be traced from end to end. At the level of the proctodeum, i.e., the posterior extremity, the tube may be seen to pulsate, widening slowly like a filling bladder and then contracting to such an extent that the lumen is obliterated. The fluid contents may be seen to move forward under impetus of the contraction of the bladder, and a form of peristaltic wave along the tube. Obviously the thickened wall of the posterior end is contractile.

While the excretory pore is visible, no fluid could be seen issuing from it as a result of contraction of the bladder. However, because of limited material, these observations are as yet inconclusive and need to be repeated.

III. DESCRIPTIONS OF NEW SPECIES

GENUS SPIRONOURA LEIDY 1856

DIAGNOSIS.—With the characters of the subfamily Kathlaniinae. Small to medium-sized worms attenuated at each extremity. Head truncate, with three low lips, sometimes partially divided. The lips each bear two bifurcate papillae, the inner branches ending in a small papillus on the inner surfaces of the lips, the outer branches ending in a larger papillus on the anterior surfaces of the lips. A pair of low flattened amphids present, one on each side of the head in close approximation with one of the anterior branches of the subventral lip papillae. Cervical papillae far posterior, nearly at the level of the excretory pore, and consisting of a projecting spherical knob.

Around the base of the vestibule is an internal ring of cuticular nature, with three nodes or thickenings, one in the middle of each lip,

serving for the attachment of the lip muscles (above) and pharyngeal muscles (below), and with three additional nodes, each one supporting an angle of the lumen of the vestibule, and thus binding the lips together.

Cuticula without alae, plectanes, or other modifications or thickenings. Transverse striations extremely minute.

Tail in both sexes conical, acute.

Muscular pharynx present, clearly separated from the esophagus. Lumen of the esophagus with accessory canals at the angles. Esophagus with a posterior bulb equipped with a mobile corrugated valve and intestinal valve (cardia), and constricted anterior to the middle, thus being divided into a small "anterior" bulb and a larger "posterior" bulb.

Intestine expanded anteriorly, gradually attenuated posteriorly, and equipped internally with dorsal and ventral ridges, resembling the typhlosole of annelids.

Proctodeum pyriform, expanded anteriorly and usually greater in diameter than the posterior end of the intestine; suspended dorsally and laterally to the dorsal and lateral lines respectively, each "mesentery" being composed of a single cell (except in the "dorsal mesentery" of the male in which there are two).

Excretory system of the "X" type, the bridge consisting of a longitudinal bar of a syncytium of three cells, under which a space giving the impression of a bladder (the pseudo-bladder).

Vulva of the female located near the junction of the middle and posterior thirds of the body. Vagina directed dorsally and anteriorly from the vulva; muscular, and lined with cuticula. Uteri opposite; ovaries with ends directed one anteriad and the other posteriad. Shell glands present between ovaries and uteri.

Male spicules variable in length, alate and acute. Gubernaculum present, sometimes incompletely cuticularized. Caudal papillae consisting of three pairs of preanal subventrals, three pairs of circum-anal subventrals, and a variable number of postanals; usually two pairs of subventrals and two pairs of laterals.

Male usually with a pseudo-sucker; sometimes four, sometimes none. Always with a series of precloacal oblique muscles posterior to the pseudo-sucker, and an accessory polymyarian muscle field between the meromyarian ventral somatics and the lateral line.

Tip of the testis near the posterior end of the body; one ascending and one descending loop to the genital tube; entire system ventral (or partially lateral) to the digestive tube. Seminal vesicle, seminal gland (cement gland), and ductus ejaculatorius present.

Parasites of turtles (usually) or other reptiles, and amphibia, and fishes (rarely).

Synonyms: *Spirura* Diesing 1861; *Falcaustra* Lane 1915; *Florencioia* Travassos 1919; *Spectatus* Travassos 1923; and doubtfully, pending further investigation, *Zanclophorus* Baylis and Daubney 1922.

Spironoura wardi n. sp.

I have made several collections of this species, which is quite distinctive from any other described species. The host is *Chelydra serpentina*. As yet I have discovered it in no other species of turtle, but in view of the variable host adaptability of other North American *Spironourans*, I judge it will be found in other hosts sooner or later. The species is named in honor of Dr. Henry B. Ward, Professor of Zoology in the University of Illinois.

DIAGNOSIS.—With the characters of the genus. Measurements in Table 2. The angle nodes of the lip support ring have characteristic horn-like points directed obliquely outward (Fig. 67). The pharyngeal supports are rather weakly developed, as are the connecting bars. There is nothing especially characteristic about the head papillae, lips, etc. Arcade glands with so little pigment as to be invisible in ordinary toto mounts. The cylindrical esophagus is thickest at about the middle. Both bulbs are almost perfectly spherical (Fig. 8). The pseudo-bladder is rather long, the posterior end about even with the middle of the posterior bulb in large specimens, farther forward in smaller specimens, and somewhat variable according to the contraction of the esophagus, body wall, etc. The pore is one-third of the length of the bladder from the anterior end.

The tip of the anterior ovary of the female lies far back of the posterior bulb, and the tip of the posterior ovary may reach the rectum. The posterior shell gland reaches to the vulva anteriorly, and the anterior shell gland lies rather far forward of the vulva. The vagina is extraordinarily long compared with spicule length (Table 2). The greatest body width of the female usually lies immediately in front of the vulva, in contrast with other species in which the greatest body width is usually near the junction of the middle and anterior thirds of the body. The body of a mature female is always abruptly narrower just posterior to the vulva.

The characters of the male are more distinctive. There are 35 to 40 pairs of muscles in the precloacal obliques, and 40 to 48 pairs in the pseudo-sucker. This is the only North American species in which the pseudo-sucker contains more muscles than the precloacal oblique system.

The male caudal papillae (Fig. 6) are arranged as follows: Two pairs of postanal subventrals are located close together about two-thirds of the length of the tail from the anus. At a level between these two is a lateral pair. Another lateral pair is located just posterior to the level of the anus.

A third pair of subventral postanal papillae is located at a level about one-third of the length of the tail posterior to the anus. Note that this pair does not occur on any other North American species, excepting *S. longispicula* (Walton) 1927.

The circum-anal papillae consist of three pairs, the anterior two of which are located very close together at the level of the anus, and the third pair slightly posterior to the anus.

There are three pairs of preanal subventral papillae, the posterior pair of which lies at about the anterior end of the cloaca, sometimes anterior to it, and sometimes posterior; rather variable in position. The middle pair always lies shortly anterior to the level of the anterior end of the spicules (when retracted). The anterior pair lies slightly more anterior to the middle pair than the middle pair is anterior to the posterior pair.

In all there are 11 pairs of caudal papillae and a single precloacal papillus in front of the anus.

TABLE 2.—MEASUREMENTS OF APPROXIMATELY MAXIMUM AND MINIMUM MATURE SPECIMENS (IN MILLIMETERS)

Spironoura wardi Mackin 1936

Females	Minimum	Maximum	Males	Minimum	Maximum
Total length.....	7.98	13.72	Total length.....	6.81	8.41
Greatest breadth.....	0.29	0.47	Greatest breadth.....	0.25	0.33
Head breadth.....	0.06	0.11	Head breadth.....	0.09	0.09
Breadth at anus.....	0.16	0.24	Breadth at anus.....	0.16	0.17
Pharynx length.....	0.06	0.07	Pharynx length.....	0.05	0.06
Pharynx breadth.....	0.07	0.08	Pharynx breadth.....	0.05	0.05
Cylindric esophagus length..	1.18	1.47	Cylindric esophagus length	0.93	1.32
Cylindric esophagus breadth	0.08	0.10	Cylindric esophagus breadth	0.07	0.08
Anterior bulb length.....	0.12	0.14	Anterior bulb length.....	0.10	0.11
Anterior bulb breadth.....	0.11	0.13	Anterior bulb breadth.....	0.09	0.11
Posterior bulb length.....	0.15	0.20	Posterior bulb length.....	0.13	0.16
Posterior bulb breadth.....	0.17	0.20	Posterior bulb breadth....	0.14	0.16
To excretory pore.....	1.00	1.38	To excretory pore.....	0.77	1.17
To nerve ring.....	0.30	0.35	To nerve ring.....	0.26	0.33
Rectum length.....	0.16	0.22	Cloaca length.....	0.12	0.17
To vulva.....	5.13	8.12	Sucker to anus.....	1.02	1.51
Vagina length.....	0.80	1.08	Spicule length.....	0.33	0.37
Tail length.....	0.53	0.79	Tail length.....	0.28	0.41
Eggs length.....	0.079	0.099	Gubernaculum length.....	0.07	0.08
Eggs breadth.....	0.059	0.060			

The tip of the testis in *S. wardi* usually lies at about the anterior end of the ductus ejaculatorius (mature specimens). The ductus is longer than usual and clearly separable into three regions.

This species is most closely related to *Spiironoura testudinis* (Baylis and Daubney) 1922. The arrangement of the male caudal papillae is almost identical in the two species. *S. wardi* differs from *S. testudinis* most markedly in possessing a pseudo-sucker, and also in having much shorter spicules.

Type host: *Chelydra serpentina*, snapping turtle.

Type locality: Southeastern Oklahoma.

Type material: In the collection of Dr. Henry B. Ward, University of Illinois, Urbana, Illinois.

Spiironoura concinnae n. sp.

Two collections of worms taken from the rectum of *Pseudemys concinna* I am designating as representing a new species. More than a hundred specimens, all of which were mature, were taken in the two collections. Mounted specimens of the same species I found in the collection of Dr. Henry B. Ward, University of Illinois, from Illinois (same host).

DIAGNOSIS.—With the characters of the genus. Measurements are given in Table 3. Unfortunately I have been able to make only two collections of this species, in both of which all specimens have been nearly of the same size range. All specimens are fully mature and may represent the approximate limit of growth. Smaller specimens are needed to complete data on the growth range and to give some idea of variations in the relative size of different organs during growth. Out of nearly a hundred specimens the females vary hardly more than a millimeter; the males, however, vary as much as five millimeters.

Head structure is shown in Figs. 68 and 69. Characters of specific value are difficult to point out, but attention may be called to the exceptional height of the lips and the very robust papillae stalks. The lips are, of course, subject to variation according to fixation and contraction of the muscles.

In the development of the arcade glands and pigmentation of the esophagus *S. concinnae* approaches *S. affine*. However, the arcade cells extend back in *S. concinnae* hardly farther than the nerve ring, while in *S. affine* they extend far beyond the ring.

Measurements of the esophagus coincide quite closely with those of *S. wardi*, but the shapes of the two bulbs are quite distinctive (Fig. 11). The posterior bulb in *S. concinnae* is more nearly pyriform than spheri-

cal, and the greatest diameter is posterior to the middle. The constrictions between the bulbs, and between the anterior bulb and the cylindrical esophagus, are shallow, so that the bulbs lack a rounded appearance.

The posterior end of the pseudo-bladder lies beside the anterior bulb and the excretory pore far forward in the bladder, about one-fourth the length of the bladder from the anterior end. In lateral view, the nuclei of the bridge cells form a triangle; the posterior two lie at about the middle, one dorsal to the other, and the third nucleus lies near the anterior end.

While in most species of *Spironoura* the measurements of the tail are not very good specific characters, in *S. concinnae* the length is extraordinary enough to warrant its use in such capacity. The relative length is considerably greater than in any other species (Table 3).

In the female the ovaries have a tendency to form a series of coils near the extremities; hence the tips are relatively far from the esophageal bulb and the rectum. The tendency to coil is not to be found in all individuals to the same degree. It is not due to crowding, since all of the body cavity space is not utilized.

In the males the tip of the testis extends posteriorly but little farther than the pseudo-sucker. A characteristic feature of the males is the very heavily developed and long ductus ejaculatorius, which extends almost to the pseudo-sucker. In a 13 mm. male this structure is 2.1 mm. long.

TABLE 3.—MEASUREMENTS OF APPROXIMATELY MINIMUM AND MAXIMUM MATURE SPECIMENS (IN MILLIMETERS)

Spironoura concinnae Mackin 1936

Females	Minimum	Maximum	Males	Minimum	Maximum
Total length.....	14.6	15.8	Total length.....	8.69	13.4
Maximum breadth.....	0.47	0.54	Maximum breadth.....	0.34	0.38
Breadth at anus.....	0.21	0.23	Breadth at anus.....	0.17	0.20
Pharynx length.....	0.07	0.07	Pharynx length.....	0.07	0.08
Pharynx breadth.....	0.07	0.08	Pharynx breadth.....	0.07	0.07
Cylindric esophagus length..	1.47	1.60	Cylindric esophagus length	1.30	1.42
Cylindric esophagus breadth	0.12	0.14	Cylindric esophagus breadth	0.11	0.11
Anterior bulb length.....	0.14	0.16	Anterior bulb length.....	0.12	0.13
Anterior bulb breadth.....	0.13	0.14	Anterior bulb breadth.....	0.11	0.12
Posterior bulb length.....	0.18	0.21	Posterior bulb length.....	0.18	0.19
Posterior bulb breadth.....	0.18	0.19	Posterior bulb breadth....	0.17	0.18
To excretory pore.....	1.36	1.42	To excretory pore.....	1.15	1.22
To nerve ring.....	0.33	0.36	To nerve ring.....	0.33	0.36
Rectum length.....	0.23	0.24	Cloaca length.....	0.26	0.28
To vulva.....	8.93	9.40	Sucker to anus.....	3.00	3.33
Vagina length.....	0.99	1.06	Spicule length.....	1.07	1.15
Tail length.....	1.02	1.25	Tail length.....	0.46	0.52
Eggs length.....	0.086	0.099	Gubernaculum length.....	0.15	0.16
Eggs breadth.....	0.059	0.066			

There are from 45 to 52 pairs of oblique muscles in the precloaca and from 25 to 32 pairs in the pseudo-sucker. Note that these figures separate this species quite definitely from *S. affine*, a closely related form.

The spicules extend only about one-third of the distance from the anus to the pseudo-sucker when retracted, and are less than half the length of the ductus ejaculatorius.

The subventral postanal papillae (Fig. 66) consist of two pairs which lie at a level two-thirds to three-fourths of the tail length posterior to the anus. At the same level is a lateral pair. A second lateral pair lies just posterior to the level of the anus.

Usually the 3 pairs of circum-anal papillae are all slightly posterior to the anal opening, although the anterior pair may be at the level of the anus or even, rarely, slightly anterior to it.

The first pair of preanals lies between the levels of the middle and anterior end of the cloaca. The second pair is rather variable, usually lying just anterior to the middle of the spicule, and sometimes forward of this point. The third pair lies anterior to the end of the spicule, sometimes only slightly so, or even with the end.

The papillae in *S. concinnae* are exceptionally small and often very difficult to see. In some specimens I have been unable to locate the anterior preanal pair, even when all the other papillae were clearly visible. It may be that this pair is actually not present in some specimens, which would not be an unusual condition.

This species has its nearest relative in *Spiromouira procera* Canavan 1929. It differs from this species in the possession of a well-developed pseudo-sucker.

Type host: *Pseudemys concinna*.

Localities: Southeastern Oklahoma; Illinois.

Type specimens in the collection of Dr. Henry B. Ward, University of Illinois, Urbana, Illinois.

Key to the North American Species of *Spiromouira*

- 1 (2) Without a pseudo-sucker.....*Spiromouira procera* Canavan 1929.
- 2 (1) With a pseudo-sucker..... 3
- 3 (6) Male with 11 pairs of caudal papillae..... 4
- 4 (5) Pseudo-sucker of the male with 40 to 48 pairs of muscles; spicules not longer than 0.37 mm. in length.....*S. wardi* Mackin 1936.
(Figs. 6, 8, 65, and 67)
- 5 (4) Pseudo-sucker weakly developed; spicules 1.2 to 1.21 mm. in length.....*S. longispicula* (Walton) 1927.
- 6 (3) With ten pairs of caudal papillae in the male..... 7

- 7 (8) Head with a series of grooves originating at the angles between the lips and spiraling part-way around the head; anterior bulb of the esophagus long, narrow, cylindrical....*S. cryptobranchi* Walton 1930. (Figs. 4, 7, and 14)
- 8 (7) Head without spiral grooves; anterior bulb inflated..... 9
- 9 (10) Spicules very long, 2.35 to 4.5 mm.....*S. chelydrae* (Harwood) 1932. (Figs. 3, 10, 12, 17, and 20)
- 10 (9) Spicules not longer than 1.2 mm..... 11
- 11 (12) Spicules not longer than 0.3 mm.; pseudo-sucker with 11 or 12 pairs of muscles.....*S. catesbeianae* (Walton) 1929. (Figs. 13, 60, 62, and 63)
- 12 (11) Spicules from 0.9 to 1.2 mm. in length..... 13
- 13 (14) Entire pseudo-bladder and excretory bridge anterior to the anterior end of the anterior esophageal bulb; 39 to 40 pairs of muscles in the ventral oblique muscles of the male.....*S. affine* Leidy 1856. (Figs. 1, 5, 61, and 64)
- 14 (13) Posterior end of the pseudo-bladder and the excretory bridge at the level of the anterior esophageal bulb or posterior to it; 45 to 52 pairs of muscles in the ventral oblique system of the male.....*S. concinnae* Mackin 1936. (Figs. 11, 66, 68, and 69)

Note: No attempt has been made to key *S. gracile* Leidy 1856 since not enough specific information is available to separate it from several other North American species.

IV. OBSERVATIONS ON GROWTH AND VARIATION

This study was undertaken primarily to test the value of formulae designed to aid in the separation of species of nematodes. Such formulae are based on measurements of various body regions or organs, and are expressed as percentages of the total body length (or width). N. A. Cobb has been the chief advocate of the use of what has become known as "Cobb's formula," and has used his own formula extensively, especially in descriptions of free-living nematodes.

GROWTH IN *Spironoura chelydrae*.—This species was selected as a basis for study for no other reason than that a wealth of material was available; certainly a necessary condition for such a study. Twenty-five specimens were measured, and each measurement computed as a percentage of total body length. The specimens were selected to cover as nearly as possible the entire size range of the species, from immature specimens to the largest mature specimen available. Only females were used, and the minimum range extended only as far as specimens in which the vulva was easily discernible. Table 4 shows the results of the measurements. The worms are numbered from I to XXV, the first being the smallest, the last the largest, and the others ranging in as nearly

evenly graded steps as possible. Only a few of the major divisions of the body were measured: the region between the anterior extremity and the level of the posterior end of the esophagus (including the bulb), the region from bulb to vulva, the region from anterior extremity to vulva, from vulva to anus, and from anus to posterior tip (tail). For each of these regions, the length in millimeters is given in the left column, and the percentage of the body length in the adjacent column to the right. At the bottom of each column of measurements is given the percentage of growth of that particular body region as computed from the smallest and largest specimens.

The first point of interest lies in the exceptionally great range in actual total size of the species. The first six specimens and the eighth specimen were sexually immature. This leaves the range of mature females from 8.68 to 22.55 mm., easily the greatest range in size of any species of *Spironoura* described. Some of the mature specimens of any other species of *Spironoura* at present described would fall within this range.

The most significant fact brought out by the measurements is the variability of percentages of different body regions to the total length. In the smallest specimen, for instance, the esophagus is 24.4 percent of the total length. In the first few immature individuals this percentage is increased, and apparently has a maximum in specimens in the 6-mm. range (27 to 29 percent of the total body length). From that point on to the maximum sized specimens the percentage decreases, until the esophagus represents only 11.7 percent of the total length. Thus while the esophagus is actually steadily increasing in size, its relative length as steadily decreases. The esophagus actually grows 105.3 percent (computed from the increase in size over the smallest specimen).

At the same time that region of the body between the bulb and the vulva grows from 2.14 mm. in length to 11.03 mm.—an increase in size of 415.4 percent. In the smallest specimen this region is 40.5 percent of the total length, and the percentage *increases* up to 48.9 in the largest specimen.

No additional discussion is necessary for other body regions. The relative position of the vulva is most constant; the tail is variable (not related to growth) to such an extent that not much may be said concerning it. The factor of most importance is that different body regions and organs very evidently do not grow at the same rate, even after the specimens are fully mature sexually. From these studies it is clear that the use of formulae based on percentages must be of very limited value. Their usefulness would depend entirely on coincidence (by different in-

TABLE 4.—MEASUREMENTS AND PERCENTAGES OF THE TOTAL BODY LENGTH OF VARIOUS BODY PARTS OF THE FEMALE
Spironoura chelydrae (Harwood) 1932

Specimen number	To posterior end of bulb		From bulb to vulva		From anterior end to vulva		From vulva to anus		Tail length		Total
	mm.	%	mm.	%	mm.	%	mm.	%	mm.	%	
I ^{1,2}	1.29	24.4	2.14	40.5	3.43	64.9	1.41	26.7	0.44	8.33	5.28
II ^{1,2}	1.84	29.5	2.43	37.7	4.27	67.2	1.58	24.9	0.50	7.86	6.36
III ^{1,2}	1.74	27.14	2.626	41.0	4.36	68.1	1.51	23.6	0.52	8.23	6.41
IV ^{1,2}	1.84	28.6	2.534	39.2	4.38	67.8	1.55	23.9	0.52	8.27	6.46
V ^{1,2}	1.79	24.8	3.055	42.2	4.85	67.0	1.90	26.2	0.47	6.56	7.23
VI ^{1,2}	1.68	19.3	4.15	48.65	5.83	67.95	2.22	25.9	0.52	6.15	8.58
VII ^{1,2}	1.84	21.1	3.80	43.8	5.64	64.9	2.38	27.5	0.66	7.60	8.68
VIII ^{1,2}	1.9	21.5	4.01	45.5	5.91	67.0	2.37	27.0	0.52	5.99	8.81
IX ²	1.9	20.0	4.33	45.36	6.23	65.36	2.64	27.7	0.66	6.90	9.53
X ²	2.03	21.1	4.17	43.4	6.2	64.5	2.64	27.5	0.76	7.91	9.60
XI ²	1.92	19.6	4.54	47.1	6.46	66.7	2.62	26.6	0.66	6.77	9.74
XII ²	1.95	18.4	4.29	46.0	6.84	64.4	3.57	28.8	0.71	6.69	10.6
XIII ²	2.00	18.5	5.31	49.0	7.31	67.5	2.82	26.1	0.68	6.34	10.8
XIV ²	1.71	14.9	5.57	48.6	7.28	63.5	3.53	30.8	0.66	5.52	11.45
XV ²	1.79	14.7	6.12	50.2	7.92	64.9	3.67	30.1	0.60	4.97	12.19
XVI ²	1.98	15.4	6.33	49.5	8.31	64.9	3.71	29.0	0.76	6.00	12.8
XVII ²	1.84	14.0	6.73	51.6	8.58	65.6	3.77	28.8	0.71	5.44	13.06
XVIII ²	2.08	14.3	7.287	50.2	9.37	64.5	4.46	30.6	0.68	4.72	14.52
XIX ⁴	2.16	13.6	7.69	48.8	9.85	62.4	4.54	28.7	1.39	8.80	15.79
XX ⁴	2.32	13.9	8.16	47.9	10.48	62.8	4.18	25.0	1.42	8.50	16.68
XXI ⁴	2.34	13.3	9.15	52.0	11.49	65.3	4.61	26.2	1.48	8.40	17.58
XXII ⁴	2.43	12.9	9.94	52.7	12.37	65.6	4.91	26.09	1.55	8.10	18.83
XXIII ⁴	2.64	13.7	10.03	52.1	12.67	65.8	5.15	26.7	1.42	7.40	19.25
XXIV ⁴	2.66	12.7	10.48	51.7	13.14	64.4	5.43	26.6	1.82	8.90	20.40
XXV ⁴	2.64	11.7	11.03	48.9	13.68	60.6	7.23	32.5	1.64	7.27	22.55
Percentage of growth	105.3	415.4	298.8	412.7	272.7	327.0

¹Immature specimens. ²From the host *Pseudemys elegans*. ³From the host *Chelydra serpentina*. ⁴From the host *Macrochelys temminckii*.

vestigators) in collecting specimens of a certain species which would be of approximately the same growth stage or size.

There is evidence that the species and size of the host may have something to do with regulating the size that a parasitic species may attain. Table 4 will illustrate this point. The smallest mature specimens of *Spironoura chelydrae* which I have been able to collect have come from *Pseudemys elegans*. Specimens VII and IX of the table came from this host (as well as some of the immature specimens). The mature individuals up to number XVIII all came from *Chelydra serpentina*. Specimens XIX to XXV all came from the alligator snapper turtle, *Macrachelys temminchii*. It is not clear whether these specimens range larger than specimens from other host species because of the difference in species, or whether the difference in size is due to the larger size of the host. All of the specimens listed in the table from *Macrachelys temminchii* were from a single host specimen which weighed approximately 100 pounds. Whatever the reason, the fact remains that the size of the parasites differs radically in different hosts.

Mention may be made here concerning the growth of the hard substances designated as "cuticular," or "chitinous." Organs of such nature are generally accepted to be secreted material and thus non-cellular. I have already called attention to the fact that these substances are not cuticular, and that they are generally understood not to be of chitin. Many workers apparently consider variation in size of such structures to be of limited nature, and the variation less than for cellular organs. They therefore use measurements of these structures more constantly than measurements of other structures, in specific diagnosis. The spicule of the male is an example. Actually such organs grow, in many cases, proportionately with cellular organs. It is not surprising that this should be so, since the size of spicules is a reflection of the activity of the cells which secrete them, and which are presumably active throughout the growth period of the individual. The advantage in use of such structures does not lie in the lack of variability in size, but in the fact that, being of hard substance, they are less liable to distortion in killing, fixation, etc.

Obviously some revision or modification of existing methods of specific description of nematodes is desirable. In order to clarify the problem, more critical separation of generic from specific characters is necessary.

I have estimated, from a thorough study of specific descriptions of North American species of *Spironoura*, that roughly 40 percent of the characters given are generic in value and thus entail that amount of wasted space in description. When such measurements, given without

due regard for variational range or maturity of specimens, are coupled with almost valueless specific characters and poor illustrations, it is quite apparent why confusion of species so commonly occurs.

V. SUMMARY

The nematode species *Spironoura chelydrae* (Harwood) 1932 (Oxyuroidea, Kathlaniidae) has been made the subject of extended anatomical studies. Special attention has been paid to the histology of the arcade system, the entire digestive tract, the genital systems, and the supportive (non-cellular) structures of the head. Analyses of the esophagus for nuclear position, type, and constancy show these considerations to be generic in extent. A new type of somatic musculature, concerning gross cell shape and arrangement, has been described.

A careful analysis of the basis for separating species by means of "nematode formulae" has shown that this method is practically valueless, and is a source of much confusion. The analysis consists of a study of growth in *Spironoura chelydrae* covering the entire range in size. Percentages of length of various body regions in all stages of growth have been computed. These percentages vary widely in the different stages, showing that growth is radically unequal when comparing different sections. It is clear that formulae for different growth stages would vary beyond the limits of usability. In addition, it is pointed out that limits of growth are determined, to some extent at least, by the host species. Lastly, contraction plays a more important rôle in variation than has heretofore been considered possible.

Two new species have been described, *Spironoura wardi* and *Spironoura concinnae*. A key for the separation of the North American species has been compiled.

EXPLANATION OF PLATES

ABBREVIATIONS

<i>a</i>amphid	<i>mc</i>muscles of the cardia
<i>ab</i>anterior bulb	<i>mr</i>muscular reticulum of the ejaculatory duct
<i>ac</i>accessor canal	<i>ms</i>median strand
<i>am</i>angle muscles	<i>msm</i>median surface muscle
<i>amu</i>anal muscles	<i>n</i>nerve cell
<i>an</i>angle node	<i>nr</i>nerve ring
<i>ao</i>anterior ovary	<i>p</i>pharynx
<i>ar</i>arcade cells	<i>pb</i>pseudo-bladder
<i>asm</i>anterior fibres of the surface muscles of the corrugated valve	<i>pc</i>primary cuticula
<i>asr</i>anterior subcuticular ring	<i>peb</i>posterior excretory bridge
<i>b</i>bar connecting angle node and pharyngeal node	<i>pg</i>preanal groove
<i>cb</i>collar of the bulb	<i>pgp</i>post-gubernacular pocket
<i>ct</i>connective tissue	<i>pn</i>pharyngeal node
<i>de</i>ductus ejaculatorius	<i>po</i>posterior ovary
<i>dg</i>dorsal gland	<i>pp</i>preanal papillus
<i>dl</i>dorsal line	<i>ps</i>pseudo-sucker
<i>ds</i>dorsal support of the rectum	<i>psm</i>posterior fibres of the surface muscles of the corrugated valve
<i>dsm</i>dorsal spicular muscle	<i>pt</i>pharyngeal tooth
<i>eb</i>excretory bridge	<i>r</i>rectum
<i>ed</i>excretory duct	<i>sc</i>spicular canal
<i>eg</i>esophageal gland	<i>sec</i>secondary cuticula
<i>ep</i>epithelial plug	<i>seg</i>seminal gland
<i>epe</i>external papillus ending	<i>sev</i>seminal vesicle
<i>epo</i>excretory pore	<i>sf</i>somatic muscle fibres
<i>gl</i>genital ligament	<i>sg</i>shell gland
<i>gm</i>gubernacular muscles	<i>sm</i>surface muscles
<i>gp</i>glandular portion of the ejaculatory duct	<i>t</i>testis
<i>gr</i>head grooves	<i>tr</i>transverse lip ridge
<i>i</i>intestine	<i>v</i>vestibule
<i>im</i>intestinal muscles	<i>va</i>vagina
<i>ip</i>internal papillus ending	<i>vag</i>vaginal glands
<i>is</i>intestinal sphincter	<i>vas</i>vaginal sphincter
<i>ll</i>lateral line	<i>vl</i>ventral line
<i>lm</i>lip muscles	<i>vs</i>ventral support cell of the rectum
<i>ls</i>lateral support cell	<i>vsm</i>ventral spicular muscles
<i>lsm</i>lateral surface muscles of the pharynx	<i>vu</i>vulva

PLATE I

- FIG. 1.—*Spironoura affine*. Anterior body region from a toto mount stained in Ehrlich's hematoxylin. Scale equals 0.1 mm.
- FIG. 2.—*Spironoura chelydrae*. Longitudinal sagittal section through the vulva, showing vaginal sphincter, vaginal gland, muscles, etc. Stained in Mallory's triple.
- FIG. 3.—*Spironoura chelydrae*. Female, from a toto mount stained in Lyon's blue. Not all of the genital tubes shown. Scale equals 0.2 mm.
- FIG. 4.—*Spironoura cryptobranchi*. Ventral view of head from a lactic acid mount. Free-hand.
- FIG. 5.—*Spironoura affine*. Anterior extremity stained with borax carmine. Details, except for arcade system, omitted. Free-hand.
- FIG. 6.—*Spironoura wardi*. Tail of male. From a toto mount stained in Ehrlich's hematoxylin. Scale equals 0.1 mm.
- FIG. 7.—*Spironoura cryptobranchi*. Tail of male. From a cleared specimen of type. Scale equals 0.1 mm.
- FIG. 8.—*Spironoura wardi*. Anterior body region from a toto mount stained in methylene blue (70% alcoholic solution). Scale equals 0.1 mm.
- FIG. 9.—*Spironoura chelydrae*. Sagittal section through the cloacal region of the male. The section is exactly in the median plane ventrally, but to one side of the median dorsally. A combination of several sections as far as the muscles of the gubernaculum and spicules are concerned. Stained in Mallory's triple. Details omitted.
- FIG. 10.—*Spironoura chelydrae*. Tail of male from a toto mount stained in methylene blue (alcoholic solution). Anterior two pairs of preanal papillae not shown.
- FIG. 11.—*Spironoura concinnae*. Anterior body region from a toto mount stained in methylene blue (alcoholic solution). Scale equals 0.1 mm.
- FIG. 12.—*Spironoura chelydrae*. Male, from a toto mount stained in indigo carmine. Scale equals 0.2 mm.
- FIG. 13.—*Spironoura catesbeianae*. From a toto mount of one of the paratypes. Scale equals 0.1 mm.
- FIG. 14.—*Spironoura cryptobranchi*. Esophageal bulb from a type specimen. Scale equals 0.1 mm.

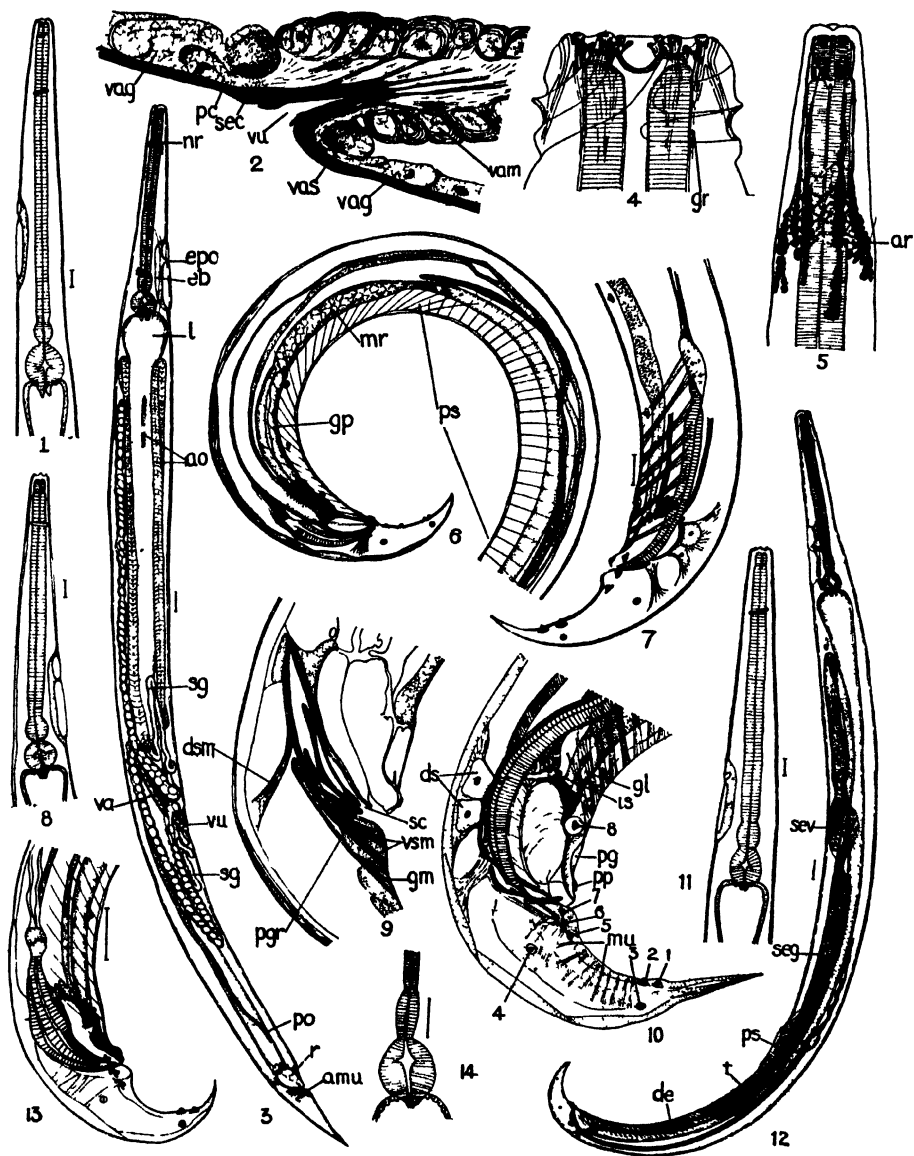


PLATE I

PLATE II

Spironoura chelydrae

- FIG. 15.—Section through the middle region of the intestine, showing dorsal and ventral ridges. Mordanted Delafield's and eosin.
- FIG. 16.—Section through the anterior esophageal bulb, showing the excretory ducts and bridge. Also nerve cells in the subventral esophageal glands of the anterior bulb.
- FIG. 17.—Face view of head cleared in lactic acid. Free-hand.
- FIG. 18.—Section through the cylindrical esophagus anterior to the nerve ring. Heidenhain's. From a single section except for the nuclei of the arcade cells. Only two of these actually appear in the section drawn; others in adjoining sections.
- FIG. 19.—Section through the rectal region of the female to show ligament cells. Delafield's and eosin.
- FIG. 20.—Ventral view of the head from a lactic acid mount. Free-hand.

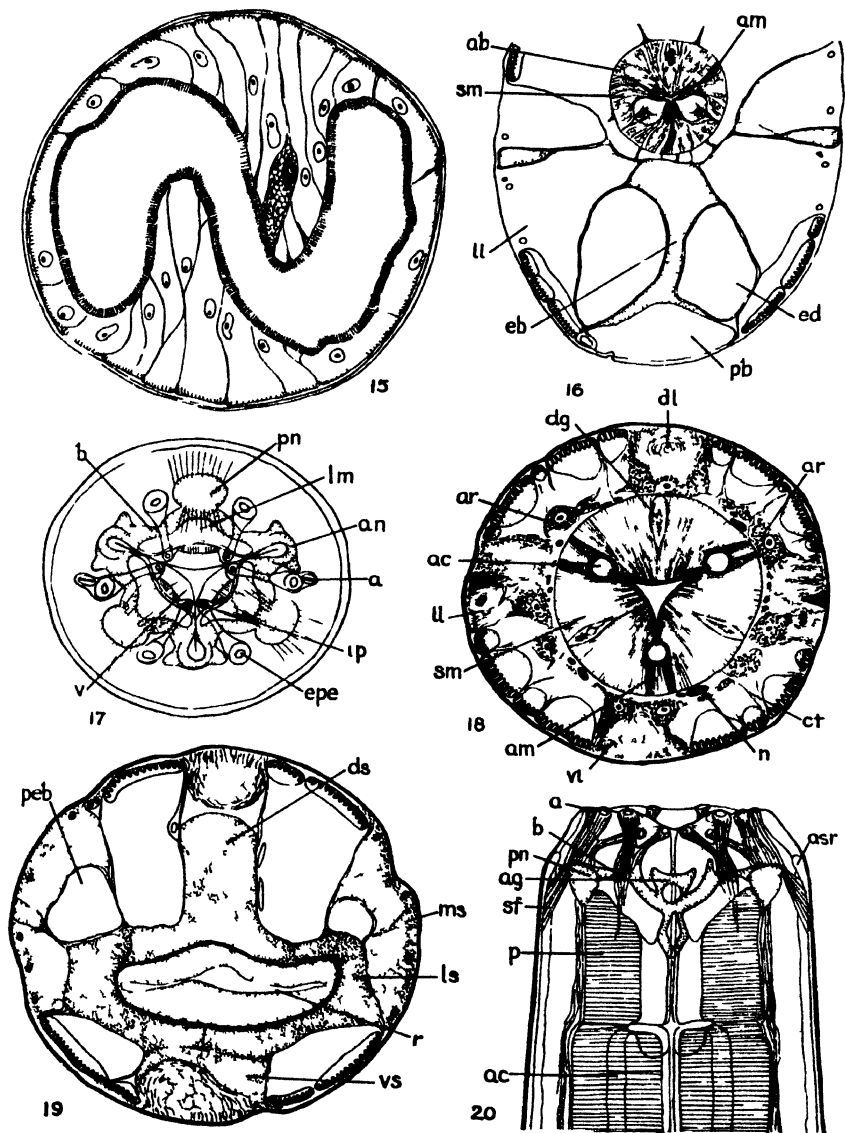


PLATE II

PLATE III

Spironoura chelydrae

- FIG. 21.—Longitudinal section of proximal end of an ovary. Mallory's triple.
FIG. 22.—Chromosomes from a fertilized egg. Heidenhain's.
FIG. 23.—Longitudinal section through the shell gland. Mallory's triple.
FIG. 24.—Longitudinal section through the genital valve and distal end of the ductus ejaculatorius. Mordanted Delafield's and eosin.
FIG. 25.—Longitudinal section through the seminal gland of the male. Mallory's triple.
FIG. 26.—Cross section of ovary near the distal end showing rachis.
FIG. 27.—Section through the genital ligament of the male.
FIG. 28.—Transverse section through the ovary; about the same region as Fig. 21. Delafield's and eosin.
FIG. 29.—Transverse section through the apical cell of the ovary.
FIG. 30.—Transverse section through the vagina.
FIG. 31.—Transverse section through the ovary in the region of the oögonia.
FIG. 32.—Longitudinal section through the apical cell of the testis.
FIG. 33.—Transverse section through the ovary near the tip, showing primordial germ cells.
FIG. 34.—Transverse section through the testis near the proximal end. Rachis present.
FIG. 35.—Transverse section through the uterus.
FIG. 36.—Longitudinal section through a portion of the wall of the ductus ejaculatorius to show secretions. Oil immersion. Heidenhain's with eosin. Free-hand.

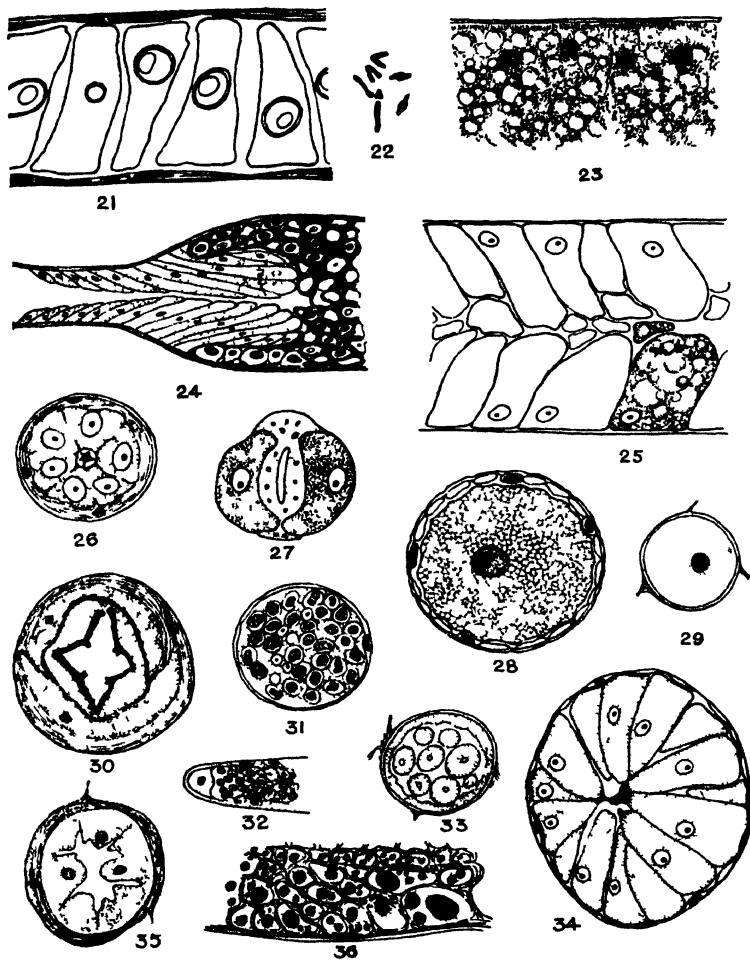


PLATE III

PLATE IV

Spironoura chelydrae

- FIG. 37.—Angle muscle nucleus of the cylindrical esophagus. Oil immersion. Heidenhain's with eosin. Free-hand. Type 3.
- FIG. 38.—Muscle nucleus from the posterior bulb. Oil immersion. Heidenhain's. Type 4.
- FIG. 39.—Surface muscle nucleus of the cylindrical esophagus. Oil immersion. Heidenhain's. Type 1.
- FIG. 40.—Longitudinal section through a dorsal cardium to show muscles and collar of the bulb.
- FIG. 41.—Transverse section through the corrugated valve.
- FIG. 42.—Surface muscle nucleus of the cylindrical esophagus and bulb. Oil immersion. Type 2.
- FIG. 43.—Transverse section through the posterior region of the intestine to show the intestinal muscles.
- FIG. 44.—Section of a spicule near the posterior end to show the gland nuclei.
- FIG. 45.—Duct of the dorsal gland. Oil immersion.
- FIG. 46.—Section of a spicule near the middle.
- FIG. 47.—Transverse section through the pharynx.
- FIG. 48.—Tangential section through the intestine to show form of the epithelial cells. Mallory's triple. Oil immersion.
- FIG. 49.—Frontal section through the posterior esophageal bulb, somewhat ventral to the middle to show musculature.
- FIG. 50.—Transverse section of intestinal cells to show stiff cilia. Oil immersion. Heidenhain's.
- FIG. 51.—Transverse section through the intestinal sphincter.

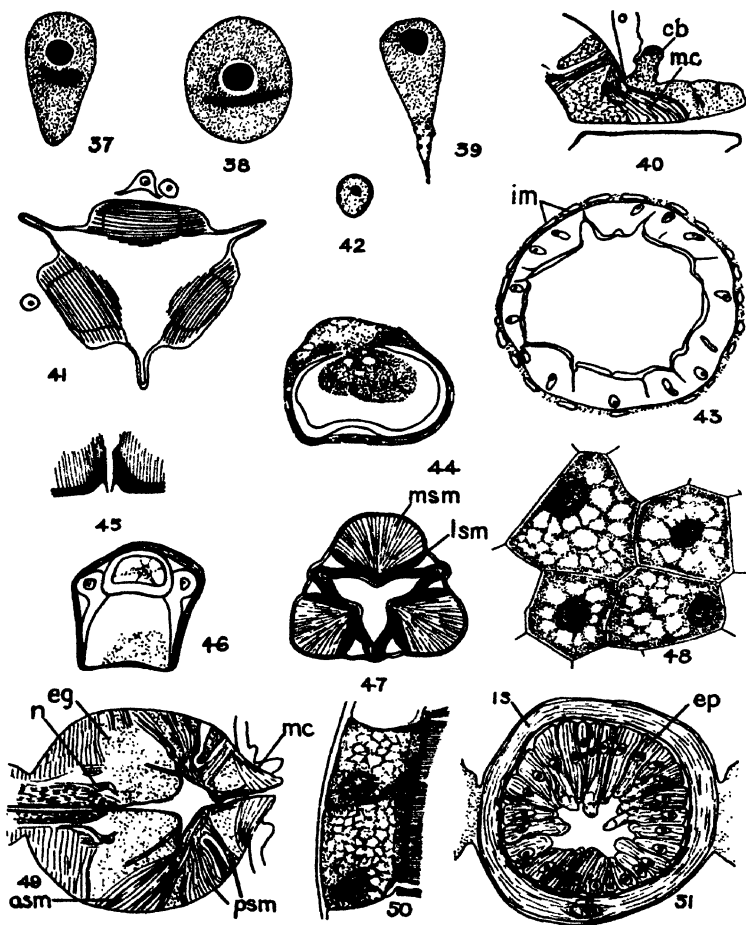


PLATE IV

PLATE V

Spironoura chelydrae

- FIG. 52.—Longitudinal section through the head and pharynx; somewhat to one side of sagittal, through the dorsal lip.
- FIG. 53.—Posterior ovarian system. Coils somewhat spread apart so that all details may be seen.
- FIG. 54.—Longitudinal section of a somatic muscle cell to show cytoplasmic inclusions. Oil immersion. Free-hand.
- FIG. 56.—Anterior ovarian system from a dissection.
- FIG. 57.—Lateral view of the angle node of the lip support ring. Free-hand from a lactic acid mount.
- FIG. 58.—The pseudo-sucker, oblique ventrals, and accessory muscle fields of the male. From a dissection stained in borax-carmin. Partly diagrammatic.
- FIG. 59.—Transverse section of a somatic muscle cell, to show the form of the contractile lamellae. Oil immersion. Free-hand.

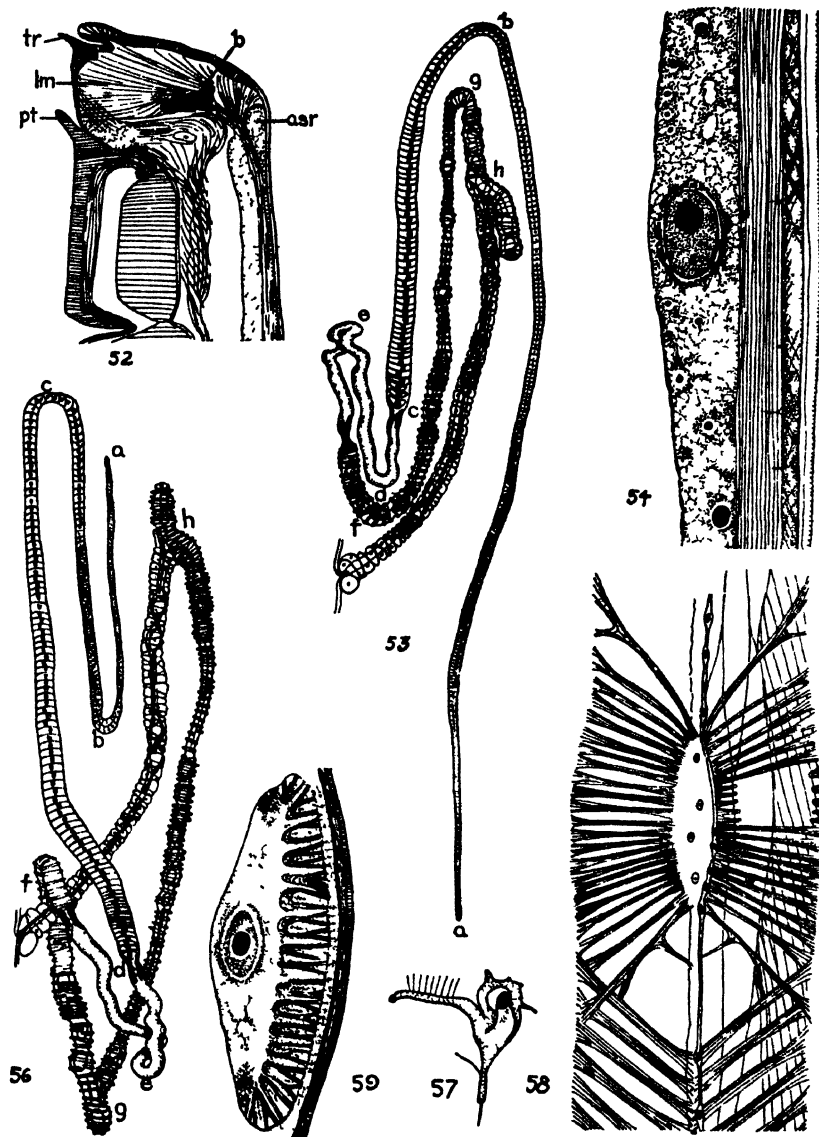


PLATE V

PLATE VI

FIG. 60.—*Spironoura catesbieanae*. Ventral view of the head from a lactic acid mount.

FIG. 61.—*Spironoura affine*. Anterior view of the head from a lactic acid mount.

FIG. 62.—*Spironoura catesbieanae*. Anterior view of the head from a lactic acid mount.

FIG. 63.—*Spironoura catesbieanae*. Anterior body region. Scale equals 0.1 mm.

FIG. 64.—*Spironoura affine*. Ventral view of the head from a lactic acid mount.

FIG. 65.—*Spironoura wardi*. Ventral view of the head from a lactic acid mount.

FIG. 66.—*Spironoura concinnae*. Tail of the male.

FIG. 67.—*Spironoura wardi*. Anterior view of the head from a toto mount in lactic acid.

FIG. 68.—*Spironoura concinnae*. Ventral view of the head from a lactic acid mount.

FIG. 69.—*Spironoura concinnae*. Anterior view of the head from a lactic acid mount.

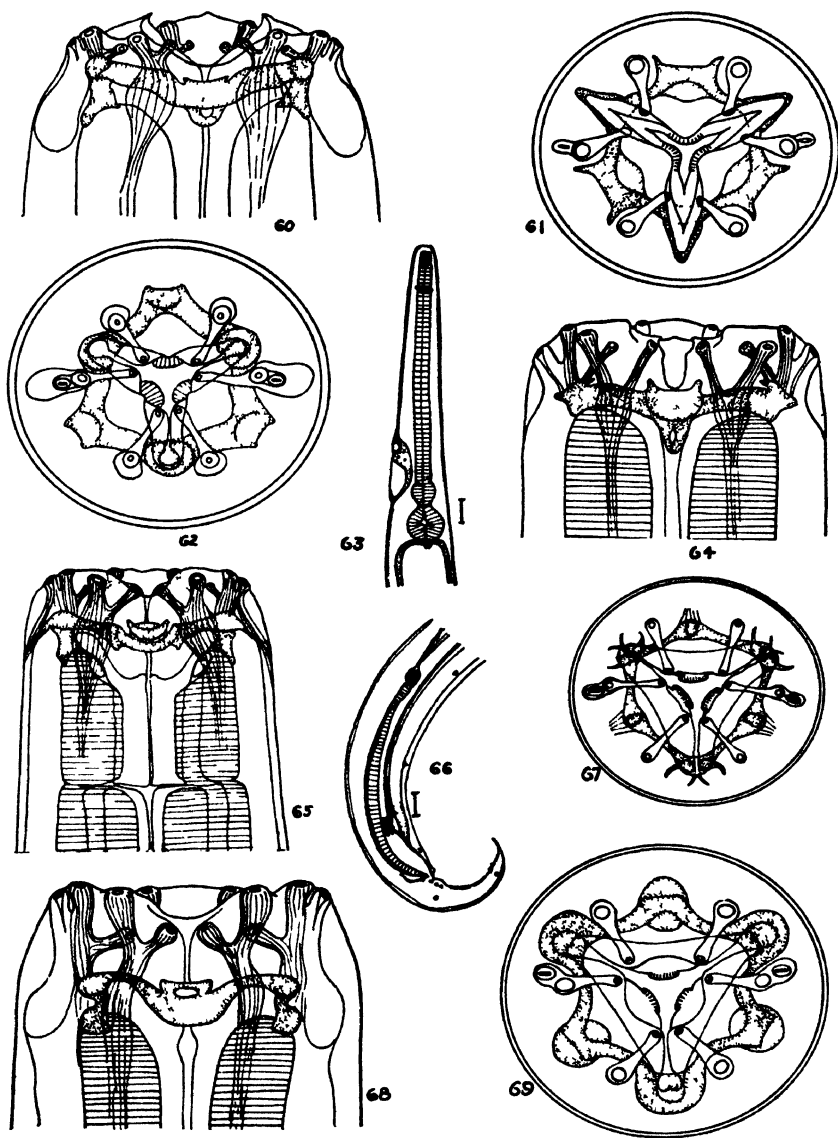


PLATE VI

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THE LIFE HISTORY OF COTYLOPHORON
COTYLOPHORUM, A TREMATODE
FROM RUMINANTS

WITH NINE PLATES

By
HARRY JACKSON BENNETT

CONTRIBUTION FROM THE ZOOLOGICAL LABORATORY OF THE
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INTRODUCTION

The knowledge of North American trematode life histories is very limited, and in many instances the life histories which have been described lack completeness. This is particularly true of the amphistomes. Cary (1909) published a life history of *Diplodiscus temperatus* which, as Cort (1915:24-30) pointed out, must be considered erroneous. Krull and Price (1932:1-37) determined experimentally the life history of this same form but omitted a description of the sporocyst. Beaver (1929:13-22) found and described all of the developmental stages in the life history of *Allassostoma parvum*, with the exception of the sporocyst. However, Beaver did no experimental work except to infest the final host. Krull (1934:171-180) obtained eggs of *Cotylophoron cotylophorum* from Puerto Rico and determined experimentally the life history of this parasite, but he did not describe any of the developmental stages.

Looss (1892:147-167) published the life history of *Diplodiscus subclavatus* (Syn. *Amphistomum subclavatum*) but he did not completely describe the miracidium nor experimentally infest the final host. He also described the miracidium of *Gastrothylax gregarius* (1896:170-177); the developmental stages of *Gastrodiscus aegyptiacus* (pp. 177-185) with the exception of the adult; and the developmental stages of *Paramphistomum cervi* (Syn. *Amphistomum conicum*) (pp. 185-191) with the exception of the adult. Takahashi (1928) described briefly some of the life history stages of *P. cervi*.

There are two methods of attack in solving trematode life history problems. One is to attempt to prove specific identity between cercaria and adult by structural comparison, and the other is to find the relationship experimentally. Several authors have described amphistome cercariae and suggested the possible relationship existing between them and known species of adults, but thus far no one has conclusively demonstrated such a relationship. In the present work the experimental method was used and all of the developmental stages were studied successively. Eggs secured from adult worms were hatched and the intermediate snail host was determined by exposing many species of snails to the free-swimming miracidia. The life history stages consisting of the egg and its development, the mature free-living miracidium, the infestation of the intermediate host, the sporocyst, the redia, the cercaria, the metacercaria, the infestation of the final host, and the development of the parasite to sexual maturity in the final host are discussed.

An attempt is made to evaluate the diagnostic value of certain morphological features which have been considered of no specific value by recent writers in extensive revisions of the classification of the amphistomes.

This report constitutes the first complete study of an amphistome life history and the first report of a representative of the genus *Cotylophoron* from the mainland of North America.

MATERIALS AND METHODS

Material for the study of the various stages of the life history of *Cotylophoron cotylophorum* was obtained from the two kinds of host of this parasite. Mature worms were collected from the rumen and immature ones from the duodenum and rumen of cows, *Bos taurus*, slaughtered at the city *abattoir* at Baton Rouge, Louisiana. The intermediate snail hosts, *Fossaria parva* and *F. modicella*, were collected from lakes, ponds, and drainage ditches in the vicinity of Baton Rouge.

Eggs deposited by worms after removal from the final host were studied alive only. Miracidia, sporocysts, rediae, cercariae, immature and mature worms were studied while alive, in toto mounts, and from sectioned material.

Miracidia were studied alive unstained or stained *intra vitam*. The *intra vitam* stains which gave the best results were methylene blue, brilliant cresyl violet, and neutral red. Fleming's osmic acid, Bouin's, or Bouin's modified with urea and chromic acid, and sublimate-acetic solution were the fixatives used but the first two were best for this material. Miracidia were stained in toto mounts with Biondi's haematoxylin and Ehrlich's acid haematoxylin. For sectioned material Ehrlich's acid haematoxylin was used most often.

Sporocysts, rediae, and developing cercariae were dissected from snails for study while alive and from toto mounts. For sectioning, the entire snail was fixed in warmed Bouin's fixative unmodified or modified with urea and chromic acid. Sublimate-acetic and modified Bouin's were used in fixing specimens for toto mounts. The stains used most often in preparing toto mounts were borax carmine, alum cochineal, and Ehrlich's acid haematoxylin. For sections the latter stain was used almost exclusively with alcoholic eosin as a counter stain.

The mature cercariae were studied alive, in toto mounts and from sectioned material. Hot sublimate-acetic solution and Bouin's were used as fixatives, but the fixed cercariae were always greatly contracted. Alum cochineal and borax carmine gave good results for toto mounts, and Ehrlich's acid haematoxylin for sections. Metacercariae were studied alive only.

Immature and mature worms are extremely resistant to external conditions and become relaxed in cold water only after several hours. The mature worms remain active from 6 to 8 hours, and the young specimens

sometimes are active after 24 hours. When relaxed the worms were placed in warmed sublimate-acetic solution or Bouin's fixative. For toto mounts borax carmine and alum cochineal gave good results, while Ehrlich's acid haematoxylin, Delafield's haematoxylin, and Mallory's triple connective tissue stain followed by eosin gave excellent results in staining sectioned worms.

The final host, *Bos taurus*, was infested by feeding metacercariae encysted on lettuce. The rate of development and the location of the parasites in the body were determined by killing and examining the hosts.

HISTORY OF THE GENUS COTYLOPHORON

Cotylophoron cotylophorum (Fischöeder, 1901) Stiles and Goldberger, 1910 was described by Fischöeder (1901:370) as *Paramphistomum cotylophorum*. His brief description is as follows:

Nur 5-8 mm lang, gedrunken, dorsoventral schwach abgeflacht. Oesophagus stark muskulös. Scharf abgegrenzter Genitalnapf. Hoden fast neben einander.

He places this species in the family Paramphistomidae Fischöeder, 1901 and in the subfamily Paramphistominae Fischöeder, 1901. Later (1903: 546-550) he redescribes this species in much greater detail.

Stiles and Goldberger (1901:15) raised the family Paramphistomidae to the rank of superfamily Paramphistomoidea, having practically the same characteristics as Paramphistomidae Fischöeder. The superfamily they divide into three families, the Gastrodiscidae Stiles and Goldberger, 1910, the Gastrothylacidae Stiles and Goldberger, 1910, and the Paramphistomidae. *Paramphistomum cotylophorum* Fischöeder, 1901 was designated by Stiles and Goldberger (1910:63) as the type species of their new genus *Cotylophoron*. They distinguish the genus *Cotylophoron* from *Paramphistomum* by a single character, the presence of a genital sucker. Fukui (1929:309) in his work on Japanese Amphistomata considers this difference not important enough to be of generic value and wishes to preserve *Cotylophoron* as a subgenus of *Paramphistomum*. I agree with Fukui and believe that *Cotylophoron* should be preserved as a subgenus only. On the other hand, Maplestone (1923:151) and Stunkard (1925: 141) consider *Cotylophoron* as a distinct genus.

Stiles and Goldberger (1910:63) described a second species for the genus *Cotylophoron* which they designated as *C. indicum*. The similarity of *C. indicum* and *C. cotylophorum* is evident from the summation of the differences between the two forms by these writers. Their statement is as follows:

Cotylophoron indicum comes close to *C. cotylophorum*, from which it differs chiefly in the structure of the oesophagus, which is provided with a bulbus thicken-

TABLE 1.—SHOWING GEOGRAPHIC DISTRIBUTION OF *Cotylophoron cotylophorum*

Host	Location	Locality of host	Author and date
<i>Bos taurus</i>	?	Togo, German East Africa	Fischhoeder 1901
<i>Bos senu</i>	?	German East Africa	Fischhoeder 1901
<i>Bos taurus indicus</i>	Stomach	East Africa	Fischhoeder 1903
<i>Ovis aries</i> (sheep).....	Stomach	India	Stiles and Goldberger 1910
"Bullock".....	Stomach	Sierra Leone, West Africa	Maplestone 1923
Bubalus sp. (buffalo).....	Stomach	Nyasaland	Maplestone 1923
<i>Aepyceros melampus</i> (nowala).....	Stomach	Nyasaland	Maplestone 1923
"Pagan dwarf bull".....	Stomach	Ilorin, Northern Nigeria	Maplestone 1923
Cobus sp. (waterbuck).....	Stomach	Zeref, Khartoum	Maplestone 1923
Bubalus sp. (hartebeest).....	Stomach	Nyasaland	Maplestone 1923
"Antelope".....	Stomach	Nyasaland	Maplestone 1923
"Antelope".....	Stomach	Rhodesia	Maplestone 1923
"Domestic cow".....	Stomach	Faradje, Belgian Congo	Stunkard 1929
"Domestic calf".....	Stomach	Belgian Congo	Stunkard 1929
<i>Neotragus pygmaeus</i> (antelope).....	Stomach	Medje, Belgian Congo	Stunkard 1929
<i>Adenota kob alurae</i> (antelope).....	Stomach	Faradje, Belgian Congo	Stunkard 1929
"Sheep".....	Rumen	Vryheid district, Natal	Le Roux 1930
"Goat".....	Rumen	Rangoon	Le Roux 1930
"Sheep".....	Rumen		
"Cattle".....	Duodenum		
	Intestine	Onderstepoort, Africa	Le Roux 1930
	Intestine		
	Duodenum		
	Rumen	Onderstepoort, Africa	Le Roux 1930
	?	Zululand, Africa	
	Rumen		
	Duodenum		
	Intestine	Zululand, Africa	Le Roux 1930
	?	Puerto Rico	Krull 1932
?	Duodenum		
<i>Bos taurus</i> (domestic cow).....	Rumen	Baton Rouge, Louisiana	Bennett 1936
<i>Ovis aries</i> (sheep).....	Rumen	Baton Rouge, Louisiana	Bennett 1936

ing in the latter species but is without it in the former. The two differ also in details of structure of the copulatory apparatus and in the position of the genital pore. In *C. indicum* the genital sucker is less sharply delimited, projects less, has a much smaller genital atrium, and the genital pore is decidedly post-bifurcal

Maplestone (1923:152-153) has pointed out that the course and length of the esophagus and the size of the esophageal thickening or bulb are subject to considerable variation in *C. cotylophorum*; and that the position of the genital pore varies in relation to the intestinal bifurcation to such an extent that neither of these points is reliable for distinguishing between *C. indicum* and *C. cotylophorum*. He further points out (p. 155) the variability in the size and appearance of the genital atrium in *C. cotylophorum* and states that the shape and number of chambers in this structure cannot be regarded as of any value for specific diagnosis. He concludes that Stiles and Goldberger were in all probability dealing with immature specimens of *C. cotylophorum*. His conclusion (p. 195) as to the diagnostic value of the copulatory structures in other amphistomes is given as follows:

. . . . the presence or absence of a prominent genital papilla, or a genital atrium, are purely matters of chance, and are of no more diagnostic value in this instance (*Gastrodiscoides hominis*) than in any other species of the group Amphistomata.

Stunkard (1925:138) attributes Maplestone's viewpoint to a confusion between physiological variations due to degrees and states of functional activity and true structural differences. On the other hand, Fukui (1929:270) agrees with Maplestone that the shape of the atrium is highly variable according to the protrusion of the genital papilla and so cannot be used for diagnosis. He (p. 319) definitely considers *C. indicum* to be a synonym of *C. cotylophorum*. I am of the opinion that the conclusions of Maplestone and Fukui concerning the importance of the genital apparatus are unsound for reasons to be pointed out later in the discussion of growth changes of *C. cotylophorum* in the final host. However, Maplestone's conclusions concerning the position of the genital pore in regard to the intestinal bifurcation and the variability in the size of the esophageal bulb are correct.

Leiper (1910:244-248) described two new species of trematodes, *Paramphistomum minutum* and *P. sellsi*, from the hippopotamus, which according to Maplestone (1923:158) should be placed in the genus *Cotylophoron*. Maplestone considers *C. minutum* and *C. sellsi* to be identical. Stunkard (1925:139) and Fukui (1929:307) accept both of them as valid species of the genus because of the large genital sucker in these forms. Regarding the validity of these two species Stunkard states:

According to the description of Leiper, *C. sellsi* is more than twice as large as *C. minutum*, the testes and ovary are about four times as large, whereas the

oral and ventral suckers are actually smaller than those of *C. minutum*. It seems incredible that these differences are mere variations and therefore I am in agreement with Leiper in regarding the two forms as distinct species.

I am of the opinion that Stunkard and Fukui are correct in considering these two species as distinct.

Only three accepted species, *C. cotylophorum*, *C. minutum*, and *C. sellsi*, have been described for the genus *Cotylophoron*.

C. cotylophorum is widely distributed as indicated by reports of this parasite from Africa, India, Puerto Rico, and the United States (present paper). The hosts from which it has been reported, its position in the host, the localities from which the hosts came, the names of the authorities reporting the parasite, and the dates of the reports are given in Table 1.

EGG

Appearance and Structure.—The eggs of *C. cotylophorum* are remarkably uniform in appearance. The shape is nearly ovoid, there being a slight attenuation at the opercular end. However, variations occur in which the eggs are completely ovoid or are more distinctly attenuated, giving a pyriform shape to the eggs (Figs. 1-11). The only marking on the shell is a small projection opposite the opercular end. This marking is usually asymmetrical in position. The operculum, which measures 22 by 3 μ , articulates with the shell by means of numerous small tooth-like projections which interdigitate with similar structures on the shell.

The eggshell is whitish when seen with the unaided eye but is transparent when seen with the microscope. In optical sections the shell is seen to be variable in thickness, being 2 μ at the operculum, 1.5 μ in the lateral areas, and 3 μ at the posterior end.

When deposited each egg contains from 40 to 50 yolk masses. Each mass is composed of a membranous envelope which is filled with a translucent liquid and numerous small granules. This material imparts to the egg its brownish-yellow appearance when studied under the microscope. The ovum, which is completely enclosed by the yolk cells, is located slightly anterior to the middle of the egg. Cleavage has not occurred in the majority of the eggs when deposited, but in some it will have advanced as far as the second cleavage stage. The outline of the ovum is easily seen in these eggs, although it is completely embedded in yolk, as is the developing embryo.

Size.—The size of the egg is of special interest because many authors attach specific significance to the size of the eggs, based on the extreme limits. Fischöeder (1903:550) in his description of *Cotylophoron cotylophorum* gave the egg sizes as 125 to 135 μ long by 65 to 68 μ wide. Stiles

and Goldberger (1910), Maplestone (1923), and Stunkard (1929) re-described the worm but did not give the egg sizes. Krull (1934:178) found the average measurement of 12 eggs teased from a preserved specimen to be 126 by 61 μ and the average measurement of 12 eggs collected from the faeces of an infested calf to be 132 by 68 μ .

Hundreds of eggs from many different specimens were measured

TABLE 2.—EGG MEASUREMENTS FOR *Cotylophoron cotylophorum*

Size of worm in mm...	3.7 x 1.3	4.5 x 2.2	6.0 x 2.0	6.6 x 2.5	9.0 x 3.0	9.0 x 3.5
Size of eggs in microns:						
1.....	121 x 58	116 x 67	126 x 62	139 x 63	134 x 63	134 x 67
2.....	125 x 67	120 x 67	116 x 67	118 x 67	122 x 67	134 x 67
3.....	125 x 67	120 x 67	125 x 67	126 x 67	126 x 67	139 x 67
4.....	134 x 67	125 x 67	125 x 71	130 x 67	126 x 67	143 x 67
5.....	115 x 68	120 x 71	125 x 71	139 x 67	130 x 67	143 x 67
6.....	137 x 68	126 x 71	125 x 71	147 x 67	134 x 60	143 x 67
7.....	137 x 68	125 x 73	125 x 71	122 x 71	118 x 71	147 x 67
8.....	116 x 71	125 x 73	129 x 71	126 x 71	122 x 71	130 x 76
9.....	138 x 71	125 x 76	129 x 71	130 x 71	126 x 71	130 x 76
10.....	125 x 76	125 x 76	133 x 71	126 x 71	118 x 71	134 x 76
Average size of eggs..	127 x 68	122 x 69	129 x 69	131 x 68	126 x 68	138 x 70

during this study and the variability was found to be much greater than that indicated by Fiscoeder or Krull. Extreme variations in size are rare, but eggs as small as 105 by 55 μ and as large as 155 by 76 μ were found. The size of 100 eggs deposited by worms over 6 mm in length varied from 113 to 143 μ in length by 66 to 76 μ in width. The size variation in these eggs which were deposited by worms that had been mature for several months was 30 μ in length and 10 μ in width. The average size of these eggs was 134 by 69 μ .

Table 2 presents data on the size of eggs produced by small, medium, and large individuals. The eggs produced by the two smaller worms averaged 124.5 by 68.5 μ and were much more variable in size than the eggs produced by either the medium or large worms. The average size of the eggs produced by the medium-sized worms was 130 by 68.5 μ while that for the largest worms was 132 by 69 μ . It is possible to conclude from these data that the average size of eggs produced by young worms is less than that of older ones and that egg sizes tend to become more uniform as age increases.

Further study of Table 2 indicates that individuals tend to produce, on an average, either small or large eggs but not both. The smallest worm, shown in column 1, produced eggs slightly larger than the worm shown in column 5. Their bodies were 3.7 by 1.3 mm and 9.0 by 3.0 mm

respectively. On the other hand, the largest worm (column 6), which measured 9.0 by 3.5 mm, produced the largest eggs, and one of the smallest worms (column 2), which measured 4.5 by 2.2 mm, produced the smallest eggs.

The average size of all these eggs in Table 2 is 129 by 68 μ , which is only a little less than that of the eggs from worms which were on an average of much larger size. This tends to support the statement that individuals produce either small or large eggs but not both.

The extreme range of variation for this group of eggs is 32 μ in length and 18 μ in width. Then by taking the extremes shown in Table 2 the egg size for this species is found to be from 115 to 147 μ long by 58 to 76 μ wide, while the average size of the eggs is 129 by 68 μ . This is approximately the same as the averages given by both Fischöder and Krull. The slightly smaller average is possibly due to the inclusion of measurements made on eggs produced by very small worms.

MIRACIDIUM

DEVELOPMENT

The development of the miracidium has been described for very few trematodes, and the descriptions which have been made vary considerably in their completeness. An accurate description of this stage in trematode development is comparatively difficult because of the minuteness and indefiniteness of the miracidial organs. Such a study necessitates both living and fixed materials which must be studied at very short intervals to determine the embryological sequence of organ development. Living material is sometimes difficult to study because of the opaque shell or the enclosed vitelline mass. Fixed materials are also difficult to study since this involves the fixation of embryos at known stages of development, sectioning and staining, followed by intensive study of the material under high magnification. Consequently only a few authors have attempted to describe this stage in the life history of trematodes.

The most complete studies of this nature were made by Thomas (1883) on *Fasciola hepatica*; Looss (1892) on *Diplodiscus subclavatus*; Looss (1896) on *Gastrothylax gregarius*, *Gastrodiscus aegyptiacus*, and *Paramphistomum cervi*; Ortmann (1908) on *Fasciola hepatica*; Johnson (1920) on *Echinostoma revolutum*; Stunkard (1923) on undetermined species of *Spirorchis*; Barlow (1925) and Ishii (1934) on *Fasciolopsis buski*; and Suzuki (1931) on *Fasciola hepatica*. Of these workers, Ortmann and Ishii used sectioned and living material while the others made their studies from living material only.

The similarity of the results obtained by these authors as to the se-

quence of organ development is remarkable. Quite naturally, however, the time of appearance of organs varies considerably because of the difference in time required for the miracida to develop under natural or experimental conditions. The slight variations found to occur in the sequence of organ development in these different species of trematodes may have several explanations: first, the difficulty with which such minute structures are recognized in either living or sectioned material; second, the almost simultaneous appearance of some organs; and, third, the lack of accurate, detailed observation.

In the present work, the development of the miracidium of *C. cotylophorum* was studied in living material only. The eggs used in making these observations were secured by taking adult worms from the host and placing them in dishes of water where they would deposit eggs for several hours. The worms were removed before they died and the water was decanted. The eggs were then washed in several changes of water in order to remove as much débris as possible. It was found that if any animal tissues were left in the dishes bacteria would destroy a large percentage of the eggs within a few days. This was demonstrated by allowing the eggs and worms to remain in the same dish until the worms had begun to decompose. In such instances only about ten per cent of the eggs would reach the hatching stage. In order to secure the highest percentages of hatching it was found necessary to change the water on the eggs at least twice each day. When the eggs were thus properly cared for about ninety per cent of them would hatch.

Time Required for Hatching.—Many eggs were obtained throughout the period from August 12, 1933, to June 22, 1934, and a record was kept as to the minimum time required for hatching (Table 3). The eggs secured between August 12, 1933, and January 4, 1934, were kept at laboratory temperatures, but the time required for the eggs to hatch increased as the winter advanced, due to the fact that laboratory temperatures fluctuated with outside temperatures, except during the day when the laboratory was heated. Between January 6 and February 23 the eggs obtained were subjected to outside temperatures at all times and none of these eggs hatched. The eggs obtained between March 28 and June 26 were again kept at laboratory temperatures, but throughout this period laboratory temperatures fluctuated day and night with outside temperatures.

No controls were established or temperature records kept for these egg-hatching experiments but the data indicate that temperature conditions are of primary importance in determining the time required for the eggs to hatch. The time required steadily increased from 15 to 29 days as the temperatures became lower from August 12, 1933, until February

23, 1934. During the period from January 6 to February 23 no eggs reached the hatching stage when exposed to outside temperatures due, it is believed, to the fact that freezing temperatures occurred a number of times. The eggs in this experiment would begin to develop, some developing as far as the ciliated stage. None was observed which had

TABLE 3.—RESULTS OF EGG-HATCHING EXPERIMENTS
Showing the minimum time required for eggs of *Cotylophoron cotylophorum* to hatch at different times of the year

Deposited	Hatched	Days elapsed	Deposited	Hatched	Days elapsed	
Aug. 12.....	Aug. 27	15	Jan. 26.....	none hatched		
Aug. 17.....	Aug. 30	13	Feb. 1.....	none hatched		
Aug. 18.....	Sept. 1	14	Feb. 6.....	none hatched		
Aug. 29.....	Sept. 10	19	Feb. 13.....	none hatched		
Aug. 31.....	Sept. 21	22	Feb. 20.....	none hatched		
Oct. 23.....	Nov. 20	28	Feb. 23.....	none hatched		
Oct. 31.....	Nov. 22	23	Mar. 28.....	Apr. 24	27	
Nov. 1.....	Nov. 23	24	Apr. 4.....	Apr. 25	21	
Nov. 8.....	Dec. 2	24	Apr. 6.....	Apr. 27	21	
Nov. 15.....	Dec. 14	29	Apr. 9.....	May 2	23	
Nov. 24.....	Dec. 20	26	Apr. 17.....	May 9	22	
Dec. 6.....	Dec. 31	25	Apr. 20.....	May 11	21	
Dec. 20.....	Jan. 14	28	Apr. 26.....	May 16	20	
Jan. 3.....	Feb. 1	29	May 29.....	June 15	18	
Jan. 4.....	Feb. 2	29	June 8.....	June 20	12	
Jan. 6.....	none hatched		June 22.....	July 3	11	
Jan. 19.....	none hatched		June 26.....	July 8	11	
Jan. 24.....	none hatched					
			Average Time Elapsed.....			21

developed beyond this condition, although living embryos were found as long as 35 days after the beginning of the experiment. The time required for the eggs to hatch during the period from March 28 to July 8 decreased from 27 days at the beginning to 11 days at the end of the experiments. This decrease in time follows steadily the increase in temperature.

In this series of experiments the time required for the eggs to hatch varied from 11 days during one of the warmest periods of the year to 29 days during one of the coolest periods, even though the eggs were kept at laboratory temperatures. The average time required for all the eggs to hatch in this series of experiments, which is overbalanced in number in the cooler months, is 21 days. In those experiments in which no eggs reached the hatching stage the temperature dropped below freezing for a part of the time. Consequently, the conclusions which may be drawn are that temperature is of great importance in determining the time required for the eggs to hatch and that freezing temperatures are fatal to them.

Developmental Rate.—A study of the rate of development of *C. cotylophorum* miracidia was made on many of the eggs used in the hatching experiments, and the results of those experiments show that the developmental rate is influenced directly by temperature. However, only the minimum time required for hatching is given in Table 3. The majority of the eggs will hatch within a few days after the minimum time but there are others which do not complete their development until months afterwards. One egg culture in which hatching began on January 14 was kept in order to determine the time required for all of the eggs to hatch.

TABLE 4.—DEVELOPMENTAL RATE OF MIRACIDIA OF *Cotylophoron cotylophorum*
Based on measurements (in microns) of different individuals at four-day intervals

No.	April 4	April 8	April 12	April 16	April 20	April 25
1.....	Diameter of ovum 18 to 25	25 x 25	55 x 38	90 x 43	154 x 38	169 x 29
2.....		25 x 25	55 x 42	65 x 46	160 x 38	153 x 32
3.....		25 x 25	59 x 42	69 x 46	101 x 42	189 x 34
4.....		25 x 25	59 x 42	101 x 46	105 x 42	197 x 34
5.....		25 x 25	59 x 42	79 x 47	118 x 42	189 x 36
6.....		29 x 29	63 x 42	90 x 47	143 x 42	168 x 38
7.....		29 x 29	50 x 46	97 x 47	103 x 46	182 x 38
8.....		29 x 29	59 x 46	72 x 49	113 x 46	193 x 38
9.....		29 x 29	71 x 46	72 x 49	105 x 50	180 x 42
10.....		34 x 34	62 x 50	80 x 50	55 x 55	210 x 63
Average.....		28 x 26	59 x 44	82 x 47	116 x 44	184 x 39

This culture was examined from time to time and after an interval of five months occasional developing embryos could be found. Other cultures were kept for varying lengths of time and this condition was observed in all of them. The cause of this slow development was not determined.

The seemingly inherent variation in the rate of development and that induced by changing temperature makes the age of the embryo an unreliable standard for determining the time of appearance of the structures of the fully developed miracidium. However, if the age and size of a sufficiently large number of embryos of any developmental series are combined a fairly reliable standard is obtained. This method was used in preference to following the development of one embryo. In this way the average rate of development can be determined, and at the same time the appearance of organs can be correlated with the age and size of any one individual.

While the developmental rate of the miracidium was studied in many eggs, only the results of observations made on one developmental series will be discussed. This series represents the average minimum time of development required by all of the eggs obtained. In making this study the eggs were observed when deposited, and cleavage was followed for

TABLE 5.—DATA ON APPEARANCE OF STRUCTURES DURING DEVELOPMENT OF MIRACIDIA OF *Cotylephoron cotylephorum*

Size in microns	Cilia	Flame cells	Primitive gut	Movement	Brain	Germ balls	Apical papilla	Penetration glands
72 x 49.....	not dev.	not dev.	not dev.	none	not dev.	not dev.	not dev.	not dev.
76 x 46.....	developed	not dev.	not dev.	none	not dev.	not dev.	not dev.	not dev.
80 x 46.....	developed	developed	developed	none	not dev.	not dev.	not dev.	not dev.
85 x 43.....	developed	developed	developed	infrequent	not dev.	not dev.	developed	not dev.
105 x 46.....	developed	developed	developed	frequent	?	?	developed	not dev.
122 x 46.....	developed	developed	developed	frequent	developed	developed	developed	developed

several hours. The eggs were then studied at four-day intervals until hatching began. Measurements were made on ten embryos selected at random at the end of these intervals in order to obtain data on the rate of growth. In addition, at the end of each interval many embryos were studied in an attempt to determine at what size and age the various organs of the miracidium made their appearance.

The data obtained on growth rate and the size at which organs can first be recognized in the embryo are presented in Tables 4 and 5.

FROM DEPOSITION TO END OF FOURTH DAY

Cleavage.—Eggs are usually deposited before cleavage begins, but occasionally they are deposited as far advanced in cleavage as the four-cell stage. The ovum or the embryo is usually situated slightly anterior to the middle of the egg, entirely surrounded by the vitelline cells, although it is sometimes peripheral in position.

The early stages of cleavage in various trematodes were described as being unequal by Ortmann (1908) and Ishii (1934). Thomas (1883) and Johnson (1920) do not describe cleavage, but their figures show that the early cleavage stages result in cells of equal size. Suzuki (1931) figures cleavage as being very regular through all of the early stages. Looss (1896) and Barlow (1925) discuss the early cleavage stages but neither their discussions nor figures throw any light on the subject. Stunkard (1923) does not mention these early stages.

The first cleavage of the ovum in this species results in two cells slightly unequal in size. As a result of the second cleavage there are one large and three small cells, one of which is considerably larger than the other two (Fig. 12). These stages occur in most of the eggs 12 hours after being deposited, but some have advanced to the eight-cell stage at the end of this time. It was impossible to determine accurately the size of the cells in the eight-cell stage due to the surrounding vitelline cells.

Size of Embryo.—The increase in size of the embryo is slight during the first four-day period of incubation. The size of 10 embryos at the end of this period is given in Table 4, column 2. At this age and size the embryo appears as a rounded, semi-transparent ball of cells, in which the nuclei vary from 3 to 5 μ in diameter. It is clearly delimited from the enclosing yolk material.

FOUR TO EIGHT DAYS

Size.—There is a marked increase in size at the end of the second four-day period, as indicated in Table 4, column 3. Some of the embryos have reached their maximum width but none of them have structures

which could be identified with certainty, other than the nuclei mentioned as being present in the earlier stages.

Vitelline Cells.—Up to this point in development there is very little change in the appearance of the vitelline cells. The original outlines of the cells are still distinct but there are fewer granules in them, giving them a more hyaline appearance.

EIGHT TO TWELVE DAYS

Size.—Between the eighth and twelfth days a difference in rate of development becomes very evident. The largest embryo observed on the twelfth day measured 101 by 42 μ while the smallest measured 65 by 46 μ , which is somewhat smaller than the largest embryo recorded at the end of the eighth day. However, the average rate of increase in size is comparable to that of the second four-day period. The majority of the embryos have reached their maximum width by the twelfth day and many structures have made their appearance.

Cilia.—The cilia are the first structures developed which can be definitely recognized, being seen on an embryo which measured 76 by 46 μ (Table 5; Fig. 5). The size of the embryos on the twelfth day (Table 4, column 4) indicates that many of them possessed cilia on the ninth or tenth day of development while there were some which had not developed them by the twelfth day.

The presence of the cilia is evidence that the epithelial cells have developed in an earlier stage but neither the nuclei nor cell boundaries could be seen at any stage in development, although the anterior and posterior limits of the cells could sometimes be seen in optical section at the lateral limits of the embryo.

Primitive Gut.—Following the cilia in development are the so-called primitive gut and two flame cells which appear at approximately the same time. These structures were first observed in an embryo which measured 80 by 46 μ (Fig. 9). The primitive gut at this stage consists of two large cells filled with granular material similar to that present in the primitive gut of the fully developed miracidium. The cells measure 17 by 11 μ and are located in the center of the body, 9 μ from the anterior end. The nucleus of each cell is 6 μ in diameter and contains a large chromatin knot which is 2 μ in diameter. The flame cells, which are located laterally and slightly posterior to the middle of the body, measure 5 by 3 μ . The ducts leading from them could not be seen.

The exact size of the embryo at which the two primitive gut cells divide to form the four cells characteristic of it in the mature miracidium was not determined but the four-cell condition was found in an individual

which measured 90 by 42 μ (Fig. 2). In this individual the primitive gut had begun to elongate, appearing similar to that in the fully developed miracidium. On the other hand, some much larger individuals (Fig. 10) possessed a gut much less advanced in development. With the appearance of the gut or shortly afterwards the apical papilla can be distinguished as a small, non-ciliated projection at the anterior end.

Muscle Tissue.—The muscle layers of the miracidium were not seen in these early stages but their presence is denoted by movement. In this series no movement was detected in any embryos under 90 by 43 μ , although it was seen in embryos of other series at a size of 85 by 45 μ . Movement in these earlier stages is very infrequent and consists of very slow and slight contractions of the anterior half of the body.

Subepithelial Tissue.—The subepithelial layer (Fig. 9) became distinguishable from the other tissues of the body during the period between the eighth and twelfth days. Not all of the nuclei of this layer can be seen, but some of them can be seen easily in optical section. They may be recognized by their characteristic ovoid shape and their position immediately beneath the ciliated epithelium. The nuclei, which are the only criteria by which this layer may be recognized, measure approximately 5 by 3 μ .

Vitelline Membrane.—According to Ortmann (1908) and Ishii (1934), working on different species of trematodes, the vitelline membrane is formed by cells which break away from the embryo during the early developmental stages and migrate to the periphery of the vitelline mass where they flatten, unite, and eventually enclose the entire vitelline mass and the embryo. In the eggs of this series the vitelline membrane was not clearly distinguishable prior to the twelfth day. Its recognition depends upon its withdrawing from the eggshell at some point, and this point is always the opercular end of the egg (Figs. 2, 3). It is then seen as a very thin membrane. The space left at the opercular end of the egg is filled with a clear liquid at first, but it is finally occupied by a viscid, granular mass called a "mucoïd plug" by Barlow (1925).

Vitelline Cells.—There is no marked change in the appearance of the vitelline cells or masses during the earlier developmental stages. There is, however, a gradual decrease in the number and an increase in the size of the masses. Perhaps this is due to the breaking down of the original masses and a subsequent coalescence of the liquids contained in them. There is also a gradual decrease in the number of vitelline granules.

The most extensive changes, in the series under discussion, occurred between the eighth and twelfth days when the vitelline masses were broken down rapidly until there were left only a few relatively large masses. However, there is no uniformity in these changes. The condition

is true for some embryos while others of approximately equal age and size still have a large number of small vitelline masses (cf. Figs. 2 and 11).

The cilia do not break down the yolk masses in this species as they do in *Fasciolopsis buski* Barlow (1925). The cilia are very seldom in motion until late in development and could scarcely be of any service in this respect. However, since they do break down shortly after most of the miracidial structures are present, it is possible that the movements of the embryo and perhaps some secretion produced by the embryo hasten this process.

Nerve Tissue.—The nervous system develops simultaneously with the primitive gut and the flame cells. It consists of many cells located on the dorsal surface of the body between the gut and the flame cells (Figs. 9, 10, 11). The nuclei are the only structures which can be seen distinctly, although there is a clear area ventral to them which probably represents the early stages of the fibrous brain.

Germinal Tissue.—The primordial germ cells can be recognized in embryos as small as 80 by 46 μ . They are massed together in the posterior third of the body, the only distinctive feature being the large nuclei characteristic of these cells. The nuclei measure 4 to 5 μ in diameter and are surrounded by very small amounts of cytoplasm.

Mucoid Plug.—The mucoid plug is not formed until after the embryo has acquired most of its organs. It was first seen in an embryo which measured 90 by 42 μ (Fig. 2), where it appeared as a granular, translucent mass at the opercular end of the egg. Barlow (1925) states that this mass is formed only at the opercular end of the egg and that perhaps it prevents embryonal secretions from loosening the operculum. This is not true in the present species since the plug may develop at either or both ends of the egg (Figs. 3, 6, 7). It becomes so viscid as development proceeds that only by extremely vigorous movements can the miracidium indent it. When present at the anterior end of the egg it forms an effective barrier between the miracidium and the operculum which has to be removed before the miracidium can hatch. I believe that this mucoid plug is nothing more than concentrated waste material excreted by the miracidium. Its positions in the egg and the fact that it does not appear until after the flame cells begin to function tend to support this belief, as does the fact that this plug increases in size and viscosity as the embryo develops. Furthermore, the concentration of this mass outside the vitelline membrane indicates that the membrane is selective and prevents the embryo from being enveloped in its excreta.

TWELVE TO SIXTEEN DAYS

After the twelfth day the only other structures to make their appearance are the four penetration glands which were first seen in an individual 122 by 46 μ (Fig. 11). Between the twelfth and sixteenth days there is a considerable increase in length and a slight decrease in width of the embryo (Table 4, column 5). Some of the embryos become longer than the egg during this period, and the posterior end of the body is flexed to provide for further increase in size (Figs. 3, 4, 6, 7).

The vitelline masses are reduced in number until there are only two large bodies which partially enclose the embryo. These are kept pressed tightly against the embryo by the vitelline membrane but move freely when the embryo moves. A few scattered vitelline granules are still present at the end of this period.

SIXTEEN TO TWENTY-ONE DAYS

Between the sixteenth and the twenty-first days there was a remarkable increase in growth. The average size of 10 embryos on the sixteenth day was 116 by 44 μ , while the average size of 10 embryos on the twenty-first day was 184 by 39 μ . There was an average decrease of 5 μ in width and an average increase in length of 58 μ . The position of the miracidium in the egg immediately prior to hatching is shown in Fig. 7.

The development of the miracidium as described here coincides in practically every detail with the development of the miracidia described by the workers mentioned earlier in this discussion. This result points to the conclusion that the chronological sequence of organ development in trematode miracidia is essentially the same.

HATCHING

The process of hatching in *C. cotylophorum* was found to be more complicated than has been described for most trematode eggs. Barlow (1925), in describing the hatching of the eggs of *Fasciolopsis buski*, states that the glands of the miracidium are of importance in effecting its release. My observations on the present species fully support this view.

No criterion was discovered which would seem to indicate exactly when the miracidium begins its hatching activities, but such efforts continue for approximately 48 hours. The mucoid plug is located at the opercular end of the egg in almost every case and is the first obstacle which has to be removed. This plug may reach a thickness of 34 μ , so crowding the miracidium in the remaining space that its body is bent almost double. No effort was made to follow in detail the formation of the structure, but, as previously stated, it makes its appearance after the

flame cells begin to function, and it is probably formed from waste materials concentrated outside the vitelline membrane. If this is true then the plug would increase in size until the miracidium begins to destroy it. If it were possible to determine at just what moment the miracidium starts to do this then it would be possible to determine at what moment hatching efforts begin.

The plug is removed or destroyed at an extremely slow rate. A number of miracidia were observed for as long as four hours each and in no case did one make any measurable progress through it. However, it is easy to find all stages of destruction of the plug in an egg culture in which miracidia are hatching.

The initial efforts consist of applying the tip of the apical papilla to the flat base of the plug, usually near its center, and then strongly contracting the circular muscles of the body. This gives the impression that the miracidium is attempting to push the plug out of the egg or to one side. It is most probable that such contraction stimulates glandular secretions and aids in forcing out the secretions. The body may contract at any point but usually the strongest contractions occur at the base of the papilla and at the junction of the epithelial plates. During such contractions the ducts of the glands become more prominent than at any other time.

The cilia apparently are of little service in hatching, although sometimes they beat energetically as the miracidium applies its apical papilla to the plug. The short, stiff cilia present on the anterior half of the first tier of epithelial plates seem to serve as a brush. At times the miracidium slightly withdraws the apical papilla and twitches its body from side to side, which may brush off parts of the plug loosened by its secretions. The miracidium is not continuously active, each period of muscular activity being followed by a slightly longer period of quiescence. Neither of these periods exceeds more than two minutes.

The plug is entirely removed after an undetermined length of time, no trace of it being found in eggs in which the miracidium is in contact with the operculum. The apparent necessity for completely removing this plug was demonstrated in one instance. In attempting to orient an egg under a cover slip by tapping gently on it with a needle the operculum was slightly loosened and a drop of water entered the egg. The plug immediately expanded until it filled approximately two-thirds of the egg and crushed the miracidium (Fig. 8). The fact that the operculum does not open as soon as the miracidium comes in contact with it is attributed to the possibility that it is cemented shut by some secretion of either the adult worm or the embryo. Many miracidia which were undergoing strong muscular contractions in an apparent attempt to liberate themselves

were observed for varying lengths of time. Occasionally one would be successful. The operculum springs back allowing water to flow in. This seems to stimulate the miracidium to vigorous activity. However, it takes the miracidium only a few seconds to find the opening and it immediately begins to squirm through. The fact that the operculum remains closed, in some instances for hours, after the miracidium comes in contact with it points directly to the conclusion that it is eventually loosened by glandular secretion and not by intermittent muscular activity.

The opercular opening is considerably less in diameter than the body of the miracidium and it takes miracidia from five seconds to twelve minutes to get out of the egg. The apical papilla, which is narrow, goes through the opening readily but the remainder of the body passes through in some instances only after a prolonged period of incessant activity. Some swim out immediately and some were observed swimming with the eggshell still attached to the posterior end.

The miracidia do not rotate or turn around in the egg under normal conditions but they do so when the opercular opening becomes filled with débris and they are unable to penetrate it. Under such conditions they remain in constant activity until death ensues. In all egg cultures about two per cent of the embryos were found developing in a reversed position. The mucoid plug in all such cases observed was present at the opercular end of the egg, but the hatching efforts of the miracidium in each case were directed against the end opposite the operculum. None was ever observed to turn around.

MATURE MIRACIDIUM

General Activity.—The miracidia hatch throughout the day and night but the numbers hatched between 8:00 and 11:00 A.M. are so much greater that the hatching may be considered periodic. Between 3:00 and 5:00 P.M. large numbers are hatched also, but relatively much fewer than during the morning hours. They can be induced to hatch in considerable numbers at any time by stirring the egg culture while under a strong light. Their positive phototropism is evidenced by the fact that they always congregate on the lighted side of a container.

After hatching the miracidia are extremely active. They usually swim in a straight line at top speed, but they follow a zigzag course at slower speeds. The body rotates in a counter clockwise direction. At other times it may contract in such a way that the anterior end of the body is pulled to one side and as a result it swims in a small circle.

Shape and Size.—When swimming straight ahead the body is pyriform, the greatest diameter being one-fifth of the body length from the anterior end. From this point the body tapers sharply anteriorly to

terminate in the small blunt apical papilla, while posteriorly the decrease in size is much more gradual, leaving the posterior end bluntly rounded (Fig. 17).

The ability of the miracidium to change its shape is quite marked. At slow rates of speed it swims along alternately contracting and extending its body, and when stopped it may contract to such an extent that it has the appearance of a slightly ovoid ball. When the anterior end is contracted the apical papilla projects from the bottom of a conical depression formed by an invagination of the body. The miracidium very often swims along in this contracted state alternately thrusting out and withdrawing the papilla while the short cilia present at the anterior end are beating rapidly. The initial impression given is that the miracidium is feeding, because particles in the water are swept down into the funnel-shaped cavity toward the tip of the papilla, but nothing was ever observed to enter the gut.

If no host is available to the miracidium it dies after 8 or 10 hours. When near death it becomes greatly distended and moves very slowly. The body becomes vesicular as the tissues begin to break down and the epithelial plates swell away from the body, but the cilia remain active for some time afterward. Motion finally ceases except for a very slow turning around in a small circle, which continues until the epithelial plates break away completely from the body. As the internal tissues break down, the nuclei float free and are concentrated in one or more groups within the body.

General Description.—Descriptions of the morphology of fully developed miracidia have been made for comparatively few species of trematodes and many of these are not complete. In view of the limited number which have been described in detail it is difficult to judge the completeness of any of the present descriptions. The miracidium of *C. cotylophorum* possesses a ciliated epithelial covering, a primitive rhabdo-coel gut, penetration glands, an excretory system, reproductive tissue, and a nervous system.

The description of the miracidium of *C. cotylophorum* presented here is based on a study of living specimens unstained and stained *intra vitam*, toto preparations, and sectioned material.

Size.—The exact size of living miracidia is very hard to determine because of their incessant activity. However, an attempt was made to measure them by using the hanging drop method. To prepare a hanging drop, one or two miracidia were pipetted onto a cover slip and the excess water was removed. A few cotton fibers were added to the remaining water. The cilia of the miracidium become entangled in these fibers and hold it in a relatively stable position without distortion of the body. It

continues to contract and extend itself but at intervals it becomes motionless. The size of miracidia measured in this way was found to vary from 153 to 210 μ in length and from 32 to 63 μ in width; the average size of 10 was 164 by 39 μ . An attempt was made to measure them first alive and then fixed, but this was unsuccessful. The vapors of warmed Bouin's and Fleming's fixatives were employed. The contraction of the miracidia fixed in this way was always abnormally great when compared with those

TABLE 6.—MEASUREMENTS OF FIXED MIRACIDIA (IN MICRONS)

No.	Length	Width	No.	Length	Width
1.....	143	38	14.....	168	32
2.....	151	38	15.....	134	38
3.....	155	38	16.....	160	34
4.....	164	38	17.....	164	38
5.....	168	38	18.....	151	42
6.....	197	38	19.....	164	34
7.....	143	38	20.....	181	38
8.....	168	29	21.....	176	38
9.....	139	42	22.....	155	34
10.....	172	42	23.....	160	38
11.....	147	46	24.....	147	34
12.....	151	50	25.....	168	34
13.....	168	46	Average.....	159.8	38.2

fixed by other methods. The size of 25 miracidia fixed by flooding them with warmed Bouin's fixative is given in Table 6. The range in size of these fixed specimens was 134 to 197 μ in length by 29 to 50 μ in width, with an average size of 159.8 by 38.2 μ . Others were fixed in warmed Fleming's fixative but there was no perceptible difference in the results produced by the two fixatives.

Epithelium.—With the exception of the apical papilla the outer surface of the body is covered by flattened, ciliated epidermal cells (Fig. 14). There are 20 of these cells arranged in 4 rows or bands which completely encircle the body. The anterior, or first, series consists of 6 cells, the second of 8, the third of 4, and the fourth of 2. Expressed by formula the arrangement is 6;8;4;2, in which the first number represents the most anterior row of cells.

The 6 cells of the anterior group cover the first fifth of the body, terminating posteriorly at the widest point in the body. The shape of the anterior part of the body is such that each cell of this group is wide at its posterior border and narrows gradually toward the anterior end, which lies at the base of the apical papilla. These cells are slightly thicker than the cells of the second group and consequently project above them very noticeably, and in slightly contracted specimens may overlap them for a

short distance. The thickness of the anterior cells is approximately $3\ \mu$ while that of the second group is 1.5 to $2\ \mu$. The cilia present on the anterior group vary from $2\ \mu$ in length at the anterior tip of the cells to $12\ \mu$ at their posterior borders. The increase in length of the cilia is very gradual. Lynch (1933:15) states that the short cilia present at the anterior ends of these cells are stiff and motionless in the miracidium of *Heronimus chelydrae*, but they are movable in the present miracidium. As pointed out in the discussion on the hatching of the miracidium of *C. cotylophorum*, they are directed anteriorly during the hatching process but in free-swimming specimens they were observed to beat in the same manner as the other cilia.

The cells of the second group are rectangular and extend slightly past the middle of the body. The cells of the third group are also rectangular but are much broader, due to the fact that there are only 4 cells in this group and the body is only slightly smaller than in the region of the second group. The 2 cells in the posterior group cover the last fifth of the body and have a triangular shape because of the body form. Their broad ends are directed forward while the tapered ends cover the tapering posterior end of the body. All the cells of the 3 posterior groups are very uniform in their thickness, which is from 1.5 to $2\ \mu$. The cilia also are very uniform in length, being approximately $12\ \mu$ in length on all of these cells. Each cilium is rooted in a distinct basal body. These bodies may be seen in living specimens as rows of very fine dots. Similar basal bodies have been described for the cilia of *Fasciola hepatica* by Ortmann (1908: 270; fig. 34a). The absence of cilia between the epithelial cells has been noted by most authors, but Talbot (1933:524; fig. 1) states that cilia cover the entire body of the miracidium of *Lechriorchis primus*. The spaces between the cells of the present miracidium are very narrow (Fig. 14), being from 1 to $2\ \mu$, but the spaces between groups of cells are very distinct and can be seen especially well in optical sections of living miracidia. These spaces are most evident between the cells of the first and second groups because of the greater thickness of the first tier of cells. Because of this thickness the cilia on the first tier of cells project further from the body, and in swimming specimens they have the appearance of a mantle or epaulets and for this reason this region of the body is sometimes designated as the "shoulder" of the miracidium.

Ciliated plates or cells have been observed by other investigators on miracidia but the numbers of cells apparently are not the same even within a single species. A survey of the literature summed up in Table 7 shows the counts for different miracidia. Looss (1892: pl. 19, fig. 17) figured these cells on the miracidium of *Diplodiscus subclavatus*. He also figured them as being present on the miracidium of *Gastrothylax*

gregarius (1896: pl. 12, fig. 121) and *Gastrodiscus aegyptiacus* (1896: pl. 12, fig. 123) but did not give the number of cells present in these forms. However, in all of them he placed nuclei representing 4 tiers of cells, and it is very probable that the number and arrangement of cells

TABLE 7.—SHOWING THE ARRANGEMENT AND NUMBER OF THE CILIATED EPIDERMAL CELLS IN MIRACIDIA

Family	Genus and species	Author and date	Arrangement and number of cells	Total cells
Paramphistomidae.....	<i>Paramphistomum cervi</i>	Sinitsein 1931	6;6;3;4;2	21
	<i>Cotylophoron cotylophorum</i>	Bennett 1936	6;8;4;2	20
	<i>Diplodiscus temperatus</i>	Krull and Price 1932	6;8;4;2	20
Echinostomidae.....	<i>Hypoderaeum conoideum</i>	Mathias 1925	6;6;4;2	18
	<i>Echinoparyphium recurvatum</i>	Rasin 1933	6;6;4;2	18
	<i>Echinostoma revolutum</i>	Beaver 1936	6;6;4;2	18
Strigeidae.....	<i>Strigea tarda</i>	Mathias 1925	6;8;4;3	21
	<i>Diplostomum flexicaudum</i>	Van Haitsma 1931	6;8;4;3	21
Schistosomatidae.....	<i>Schistosomatium douthitti</i>	Price 1931	6;8;4;3	21
Fasciolidae.....	<i>Fasciola hepatica</i>	Thomas 1883	4-5;5-6;3;4;2	18-20
	<i>Fasciola hepatica</i>	Coe 1896	6;6;3;4;2	21
	<i>Fasciola hepatica</i>	Ortmann 1908	6;6;3;4;2	21
	<i>Fasciola halli</i>	Sinitsein 1931	6;6;3;4;2	21
	<i>Fasciola californica</i>	Sinitsein 1931	6;6;3;4;2	21
	<i>Fascioloides magna</i>	Sinitsein 1931	6;6;3;4;2	21
	<i>Fasciolopsis buski</i>	Barlow 1925	6;6;6;6;6	30
Troglotrematidae.....	<i>Paragonimus westermani</i>	Ameel 1934	6;6-7;3;1	16-17
Heronimidae.....	<i>Heronimus chelydrae</i>	Lynch 1933	4-6;6-10;3-6;1-2	16-22

in each miracidium is 6;8;4;2. Unfortunately, he did not figure the nuclei of these cells in the miracidium of *Paramphistomum cervi* (1896: pl. 12, fig. 125). Sinitsein (1931) has given the epidermal cells of the miracidium of *P. cervi* as 6;6;3;4;2, which is very different from the number of cells described as being present in other miracidia belonging to

the family Paramphistomidae. It is probable that Sinitsin was mistaken in the number of epithelial cells in this form, doubtless being influenced by the number of cells present in the other miracidia which he has apparently described correctly. We may then assume that the number of epidermal cells for the family Paramphistomidae may be expressed by the formula 6;8;4;2.

The formula for the epidermal cells of the Echinostomidae miracidia based on the same number and similar arrangement which has been described for the different species may be expressed as 6;6;4;2. On a similar basis the formula for the Strigeidae miracidia may be expressed as 6;8;4;2, and for the Schistosomatidae miracidia as 6;8;4;3. The Fasciolidae miracidia formula might be expressed as 6;6;3;4;2 were it not for the extremely aberrant number of cells described for the miracidium of *Fasciolopsis buski*. The cells of the Troglotreumatidae and of the Heronimidae miracidia are not uniform in number for the single species described for each of these families.

Price (1931:703) has pointed out the possibility that the number and arrangement of the ciliated epidermal cells may be of some value in establishing relationships between families or larger groups. As she has said, the number of these cells possibly indicates relationship between the Strigeidae and the Schistosomatidae. Lynch (1933:10) has expressed doubt that these cells are of any value in determining relationships of various groups. However, the preponderance of the evidence points to the conclusion that the number and arrangement of these cells may be of value for establishing relationships within the families, and there is some evidence that it may be of value in establishing relationships between families. A final conclusion cannot be based on the small amount of information now available.

The nuclei of the epidermal cells were studied in living specimens stained with *intra vitam* stains, in stained toto mounts, and in stained sectioned material. The nuclei of the first group of cells are irregularly cylindrical in shape and are located near the posterior borders of the cells. The nucleus in each cell measures 7 to 10 μ by 2 to 3 μ in surface view. The shape of the nuclei of the second group of cells is so irregular that it may be described as being lobed. These nuclei also are located near the posterior borders of the cells and measure approximately 6 by 3 μ . The nuclei of the third group of cells are similar in appearance to those in the first group, although they are somewhat larger, being 9 to 12 μ by 2 to 3 μ in size. They are located very near the posterior boundaries of the cells. The nuclei in the last group of cells are found very near the anterior borders of the cells. These nuclei are larger than any of the others, measuring 12 by 3 μ , although their shape is much the same as

that of the nuclei in the first and third groups. The positions and shapes of the nuclei of all these cells as seen in cross sections are shown in Figs. 18, 19, 20, 21. The thickness of the nuclei as seen in cross section is about 1 μ .

The nuclei of the epidermal cells have been described for only a few miracidia. Thomas (1883: pl. 2, figs. 5, 6) figures them in the miracidium of *Fasciola hepatica* as round and located in the posterior part of the cells; Leuckart (1886:63; fig. 37) figures them as round and centrally located; Ortmann (1908: table 14; fig. 38) figures them as round in sectioned material and located toward the posterior border of the cells; Coe (1896:565) was the first author to describe in detail their shape and position. Sinitsin (1931:426; fig. 8) describes and figures these nuclei as being long and located at the posterior borders of the cells in the miracidia of *Fasciola halli*, *F. californica*, *Fascioloides magna*, and *Paramphistomum cervi*. Lynch (1933:20) saw them in the miracidium of *Heronimus chelydrae* as circular and flat in cross section. He could not see them in surface views and his statement as to their shape is open to question, since one might easily gain the impression that they are round when seen in sectioned material only. Krull and Price (1932:5; fig. 6) have described nuclei for the miracidium of *Diplodiscus temperatus* which closely resemble those of the miracidium of *C. cotylophorum*. The chief difference is that the nuclei of the second and third groups of cells are more anteriorly placed in the miracidium of *D. temperatus*.

Subepithelium.—The subepithelium is a thin, transparent layer located immediately beneath the ciliated epidermal cells and is continued forward at the anterior end to form the apical papilla (Fig. 17). The cell boundaries of this layer could not be determined but its extent is clearly indicated by the nuclei. The layer varies in thickness with the state of contraction of the miracidia, but in well extended specimens it is approximately 5 μ thick. It is somewhat less than this in the posterior region of the body, which is distended by the germ mass, and in the region of the body distended by the primitive gut and the brain. Slightly anterior to the middle of the body, between the brain and germinal tissue, there is an inward protrusion of this layer in which the flame cells are embedded. The nuclei are elongate or almost round and contain a number of small chromatin masses (Fig. 22) which aid in distinguishing these nuclei from the others of the body. The size of these nuclei is 4 to 6 μ by 3 to 4 μ . Krull and Price (1932:6; fig. 7) described and figured the nuclei of this layer as round and showed that they are arranged in 3 definite rows encircling the body of *Diplodiscus temperatus*. No other authors have described the nuclei of this layer as round or attempted to demonstrate that they are arranged in definite positions in the body.

In the present work a careful study of these nuclei was made and it was found that they vary from elongate to round in shape and that they are distributed in 4 principal groups (Fig. 15). However, these 4 groups do not contain all of the nuclei. There are many nuclei situated irregularly throughout the subepithelium, and a careful count of all the nuclei indicated that the number is far from constant. The first group, which is located beneath the anterior limits of the second group of ciliated cells, consists of from 10 to 20, with an average of 16. The second group is located in the middle of the body and the number of nuclei varies from 12 to 19, with an average of 15. The third group is located immediately beneath the termination of the third group of ciliated cells and variation in this group was found to be from 6 to 11, with an average of 8. The fourth group, consisting of from 2 to 4 nuclei, with an average number of 3, is located in the posterior extremity of the body. Some of these nuclei were occasionally seen undergoing mitosis, indicating still more the futility of attempting to determine the number of cells in the subepithelium. The presence of dividing nuclei in this layer indicates that the miracidium may grow while free-living but no experiments were made to determine the correctness of this supposition.

Muscle Tissue.—The rapid and strong contractions and extensions of the miracidium indicate a well developed musculature. The muscles observed in this miracidium were the circular and longitudinal layers which are between the ciliated epidermal plates and the subepithelium. The layer of circular muscles is located external to the longitudinal layer and the two are pressed closely together (Fig. 23). The circular muscles can be seen in living specimens and sectioned material as minute parallel bands closely set together. A single band or fiber measures approximately $1\ \mu$ in diameter and the distance between fibers is about $1\ \mu$.

The longitudinal muscles are slightly more developed than the circular muscles in the anterior region of the body. These muscles are arranged in parallel bands also. Each band measures approximately $1.5\ \mu$ in diameter, and the distance between bands is about equal to their diameter. The greater development of the fibers at the anterior end of the body is due, perhaps, to the fact that they serve to retract and extend the apical papilla and the anterior region of the body.

The muscles in the posterior regions of the body are extremely difficult to demonstrate but their presence is indicated by the ability of the miracidium to extend and contract this region. The circular muscles can be seen in living and stained toto mounts and the arrangement is the same as in the anterior region of the body. The longitudinal muscles were never seen clearly in this region of the body.

The arrangement of muscles in miracidia has been described by several

authors and there is close agreement between their descriptions. Ortmann (1908) describes and figures the muscles of the miracidium of *Fasciola hepatica* as having an arrangement very similar to that of *C. cotylophorum*. Looss (1892, 1896) describes and figures the muscles of the miracidia previously mentioned as being the same as in the closely related miracidium described here. Reisinger (1923:12; fig. 3) describes and figures the muscles of the miracidium of *Schistosoma haematobium*. He describes the circular muscles as bands 0.6 by 0.1 μ placed at intervals of 0.8 to 1.1 μ . The longitudinal muscles are similarly arranged but measure only 0.5 μ in breadth and are placed at intervals of 2 to 4 μ . The muscles of this miracidium are much smaller than those of *C. cotylophorum* but the two agree as to arrangement. The retractor muscles described by Reisinger as being present at the anterior end of the body could not be seen. Ishii (1934:30) describes large muscle cells containing nuclei in the miracidium of *Fasciolopsis buski* but similar structures were not seen in the present material.

Primitive Gut.—The structure usually called the primitive gut by writers in their descriptions of miracidia is a flask-shaped structure of variable proportions, depending on the state of contraction of the miracidium. In elongated specimens it may extend slightly posterior to the middle of the body, while in contracted specimens it becomes broadened and occupies all of the available space in the anterior fourth of the body. In specimens of average extension it extends almost to the center of the body. It terminates anteriorly in a narrow duct which does not open to the outside. The coarsely granular contents first appear when the gut consists of only two cells (Fig. 9). Later the two original cells divide and the four resulting cells apparently become confluent, because no cell boundaries can be found. The nuclei remain in the posterior part at all times and are surrounded by cytoplasm which stains more darkly than any other region of the gut. The granular contents move freely and completely fill the gut with the exception of the anterior tip of the duct near its termination in the apical papilla. The nuclei are easily recognized in all preparations of the miracidia because of their position and size. They measure 5 to 7 μ in diameter and contain several small masses of chromatin, usually grouped together near the center of the nucleus.

The development of the primitive gut at some distance from the anterior end of the body, the size of the cells and their nuclei, the early development of the granular contents, the absence of a definite cell wall around each nucleus after the four-cell stage is reached, the concentration of cytoplasm around the nuclei at the posterior end of the gut, the absence of a mouth and a lumen, and the complete disappearance of the contents immediately after penetration of the miracidium into the snail

host while the nuclei may still be identified—all give evidence in favor of interpreting this structure as being a gland rather than a gut.

The earlier writers—Schauinsland (1883), Thomas (1883), Leuckart (1886), Looss (1892, 1896), Coe (1896), and Ortmann (1908) as well as some of the later writers—Faust and Meleney (1924), Mathias (1925), Barlow (1925), Sinitsin (1931), and Van Haitsma (1931)—considered this structure as a primitive or vestigial gut. More recently, Reisinger (1923), Manter (1926), Price (1931), and Lynch (1933) have presented evidence in favor of considering this structure as a gland. An analysis of the opinions of these writers leaves some doubt as to the nature of this organ, but I believe, for the above reasons, that it is a gland and that it functions during the penetration of the miracidium into the snail.

Penetration Glands.—These glands have been described for many miracidia, but within the family Paramphistomidae they have been observed only in the miracidium of *Diplodiscus temperatus* by Krull and Price (1932:7; fig. 7). Looss (1892, 1896) does not describe them for the miracidia of the forms previously mentioned, all of which belong to this family. Krull and Price found two pairs of these glands, a pair being situated on each side of the gut. In *C. cotylophorum* there are two pairs of these glands which are extremely hard to detect. However, they may be observed late in the development of the miracidium as well as in the mature specimens (Figs. 11, 17). They are filled with a clear non-granular substance which is difficult to stain. They extend posteriorly for about one-fifth of the body length from their openings at the base of the apical papilla. The nuclei of these cells, which are located near their posterior ends, measure 4 to 5 μ in diameter.

Nervous System and Sense Organs.—The nervous system consists of a central fibrous mass, nerves, and nerve cells. The central fibrous mass is located dorsal to the posterior part of the primitive gut, where it may be seen easily in both living and stained specimens surrounded by the nuclei of the nerve cells (Fig. 22). In mounted specimens the brain appears to be lateral in position, due to the pressure of the cover slip (Fig. 17). It is oval or quadrangular in shape when viewed from the dorsal side and measures approximately 20 by 25 μ . It is from 14 to 16 μ in depth and characteristically forms an indentation in the dorsal side of the primitive gut, which can be seen in lateral view.

No nerves passing out from the brain could be seen in living specimens stained *intra vitam* or in stained toto mounts. It was in sectioned material only that large fibrous structures resembling nerves were found arising principally from the lateral surfaces of the brain, although smaller fibers were found arising at various other points. Two large

fibers were found passing obliquely forward from the brain to the lateral processes located between the first and second rows of ciliated epidermal cells (Fig. 22). While similar processes or nerves were observed to leave the brain from its posterior lateral surfaces, they could not be traced to their terminations. Neither could the smaller nerves be traced to their terminations, although finer striations observed in longitudinal sections of the anterior part of the body may have been nerves passing to terminations in this region.

The nerve cell boundaries were not seen at any time but their nuclei are stained readily by *intra vitam* stains and by other stains used on sectioned material. These nuclei are located in largest numbers immediately anterior to the brain mass, although a few were found scattered completely around it. These nuclei were found to vary from 12 to 20 in number while the average number was 17. No nerve connections could be traced to or from them. In appearance they greatly resemble subepithelial nuclei but they are round and slightly smaller, and the dense chromatin causes them to stain more darkly. They vary in size from 2.6 to 3.4 μ in diameter.

Looss (1896: pl. 12, figs. 119, 121, 125) has figured a central fibrous nerve mass surrounded by nuclei for the miracidia of *Gastrothylax gregarius*, *Gastrodiscus aegyptiacus*, and *Paramphistomum cervi* which is very similar to that of the miracidium of *C. cotylophorum*. He also observed anterior and posterior nerves arising from each side of the fibrous nerve mass in the miracidium of *Gastrothylax gregarius*. Krull and Price (1932) described a central mass for *Diplodiscus temperatus* but did not find the anterior and posterior nerves. However, they did describe 6 nerve cells located anterior to the brain which had fibers connecting the brain and the tip of the apical papilla. These cells were not observed in the present species. Reisinger (1923:16; fig. 3) in his description of the nervous system of the miracidium of *Schistosoma haematobium* found structures similar to those found in the present material, as did Lynch (1933:22; fig. 9) in the miracidium of *Heronimus chelydrae*.

The only sense organs observed on the miracidium of *C. cotylophorum* were a pair of structures which have been variously named anterior ducts, anterior papillae, mucoid secretion, lateral papillae, and lateral processes by different authors. These organs are located laterally between the first and second rows of ciliated epidermal cells (Fig. 14). Each papilla appears as a clear structure, approximately hemispherical in shape. It was difficult to make out any internal structures connected with these papillae but at times in living miracidia there appeared to be a duct extending inwardly and posteriorly from each papilla which seemed to terminate in a small vesicle in the region of the central nerve mass.

This structure is probably the large nerve previously described as passing from the brain to the lateral papilla.

Cort (1919:516) and Faust and Meleney (1924:28) called these papillae anterior or lateral ducts and stated that the extrusion of substances from these structures was observed. Stunkard (1923:183) made similar observations on the miracidia of *Spirorchis*. The present observations agree with those of Sewell (1922:287) who found no openings in these structures in the miracidia of *Cercariae Indicae* xv. Price (1931:705) made similar observations on the miracidia of *Schistosomatum douthitti*. Living miracidia of *C. cotylophorum* were observed for great lengths of time and even when subjected to pressures great enough to break the body walls no substance was observed to be extruded.

Looss (1896: fig. 125) figures but does not discuss two small lateral papillae on each side of the miracidium of *Paramphistomum cervi*. Krull and Price (1932: fig. 7) figure one lateral papilla on each side of the miracidium of *Diplodiscus temperatus* but they show no structures in connection with them nor do they suggest the possible function of these papillae.

That these structures are sensory in *Schistosoma haematobium* was clearly demonstrated by Reisinger (1923) who has described and figured nerves passing from the central nerve mass to them. Lynch (1933:31; fig. 9) has demonstrated in a similar way that these papillae are sensory in the miracidium of *Heronimus chelydrae*. The present observations agree in detail with those of these two workers and I believe that these structures are sensory.

Many other structures designated as sensory in function have been described for other species of miracidia by Coe (1896), Ortmann (1908), Price (1931), Reisinger (1923), and Lynch (1931) but none of these were found in the present material.

Germinal Tissue.—The germinal tissue was studied from its first appearance in the developing embryo and the conclusions arrived at are a result of detailed study on living material, stained toto mounts, and serial sections. In the youngest forms in which the germinal tissue could be distinguished it had a homogeneous translucent appearance. Nuclei 4 to 5 μ in diameter were present. As the miracidium develops some of the germ cells break loose into the central cavity. One large and several small germ balls usually are present when the miracidium is hatched, although some are hatched in which no definite germ balls have developed. In the latter cases there are many germ cells which seem to be free in the central cavity. The posterior three-fifths of the body is completely filled by the germinal tissue in the fully developed miracidium.

There is a pronounced difference in the appearance of this tissue and

the enclosing subepithelium in stained specimens. The nuclei of the subepithelium of this region are ovoid and do not stain as darkly as do the round nuclei of the germinal tissue. No cell boundaries could be distinguished but the limits of the germinal tissue could be established by the numerous large, closely packed nuclei, the granular appearance of the tissue, and its much greater affinity for stains than the subepithelial layer. The germinal nuclei, representing the germ cells, form a thick layer in the posterior extremity of the body and are located also around a central cavity which extends forward past the middle of the body (Fig. 16). These nuclei, which measure 4 to 5 μ in diameter, have numerous small scattered chromatin masses and one large centrally located mass. The amount of cytoplasm surrounding each of these nuclei is very small. After being liberated into the central cavity the germ cells develop into germ balls by unequal cleavage (Fig. 17). Early in cleavage a thin membrane encloses the developing germ ball. This membrane is present around each of the germ balls and it seems to develop from very small cells located in the periphery of the germ balls. Ortmann (1908:287) discusses a similar structure in the miracidium of *Fasciola hepatica* and Lynch (1933:29) has done the same for the miracidium of *Heronimus chelydrac*. The observations made by Looss (1892), Price (1931), and Lynch (1933) that the germ balls are held in position by fiber-like attachments were not confirmed in the present material. Germ cells located in the posterior part of the body at the end of the central cavity were observed to be attached to the lateral germinal areas by extensions of the cells but no connections were found for the cells which were free in the cavity or for the germ balls.

Excretory System.—The excretory system is very similar to that described in miracidia of many species. It consists of two laterally situated flame cells and their ducts (Fig. 15). The flame cells are located in the previously mentioned protrusion of the subepithelial layer, immediately anterior to the middle of the body. Each flame cell measures approximately 10 by 3 μ . Reisinger (1923) in his detailed study of the excretory system of the miracidium of *Schistosoma haematobium* states that a flame cell nucleus is lacking. There are several nuclei embedded in the tissue surrounding the flame cells of the present material, but since cell boundaries were not seen no nucleus could be definitely associated with the flame cell. However, a nucleus was found in close proximity to the basal plate of the cell (Fig. 13) which is probably directly associated with it. The excretory tubule for each flame cell extends posteriorly in loose coils almost to the excretory pore. Here it loops back to the flame cell where it again turns on itself and extends to the excretory pore which is located laterally between the third and fourth epidermal plates.

Immediately before reaching the pore the tube is expanded to form a small excretory bladder. Krull and Price (1932:8; fig. 8) describe two duct nuclei for each of the tubules in the miracidium of *D. temperatus*, but these nuclei could not be found in the miracidium of *C. cotylophorum*.

INTERMEDIATE HOST

DETERMINATION OF THE HOST

Two methods were used in determining what snail or snails would serve as the intermediate host of *C. cotylophorum*. The first consisted of searching known foci of infestation for infested snails, and the second consisted of exposing several species of snails to the free-swimming miracidia followed by dissection of the snails after an interval of 10 or 15 days to discover whether or not there were any developmental stages present in them.

Natural Infestation.—The distribution of naturally infested snails depends entirely upon the distribution of infested carriers of the adult worm. Consequently, in order to determine what region of the country surrounding Baton Rouge, Louisiana, had the largest number of carriers, many of the cattle slaughtered at the city *abattoir* during the period from June 6, 1933, to June 27, 1934, were examined for these worms. When an infested cow was found, the range from which it came was located and searched for the intermediate host of *C. cotylophorum*. It soon became evident that the worms were more abundant and more frequently found in cows which came from the low, semi-swampy ranges located south and east of the city. Occasionally cows from the hilly ranges north of the city were found to be infested and upon examination of the ranges from which these came it was found that they had access to either a small pond or lake or to a stream which ran through open fields. It was not until May 26, 1934, that a natural infestation was found in specimens of *Fossaria parva* taken from the margin of an artificial pond southeast of Baton Rouge.

Experimental Infestation.—The presence of strongly developed cilia combined with the swimming ability of the miracidium gave basis for a surmisal that the intermediate host was either an aquatic or amphibious snail. Several genera of snails are commonly present in or around streams, lakes, and ponds in this region. Prior to the finding of naturally infested intermediate hosts, snails were collected from an area semi-circular in shape with a radius of approximately 20 miles, being taken in every instance from a range on which the cattle were known to be infested with *C. cotylophorum*. The snails were examined first by placing

them in water to determine whether or not fully developed cercariae were present, and then by dissection for the earlier developmental stages. When found not to be infested others were then exposed to free-swimming miracidia. The snails used in these experiments were *Physa halei*, *Helisoma lentum*, *Succinea retusa*, *Succinea unicolor*, *Fossaria parva*, *Fossaria modicella*, and undetermined species of *Physa* and *Campeloma*. Of these only *Fossaria parva* and *F. modicella* could be infested experimentally.

F. modicella was found in only one locality near Baton Rouge, Louisiana, late in the spring of 1934, and consequently is not used in the following discussion on the development of larval stages of *C. cotylophorum*. Snails of this species collected from near Urbana, Illinois, by the writer and from Turkey Run State Park, Indiana, by Dr. H. J. Van Cleave were infested experimentally also. Krull (1934:171) secured this same species from Utah and was able to infest these snails with the miracidium of *C. cotylophorum*. *F. parva* was found frequently and doubtless is the species which serves most often as the intermediate host of this parasite in the vicinity of Baton Rouge.

BIOLOGY OF *Fossaria parva*

Fossaria parva is a small amphibious snail which rarely exceeds 7 mm in length. It is commonly found near the margins of small ponds, lakes, and streams where there is some decaying vegetation which it uses for food. The snails were rarely found in the water, and they are very helpless when caught by any current, being carried along until they drift against some object. They were never found in areas which were shaded at all times. The normal habitat is a well moistened area which is subject to direct sunlight for the greater part of the day.

The snails were found in largest numbers along the margins of an artificial pond which served as a source of drinking water for cattle, and along the margins of a large unshaded drainage ditch. Observations were made at intervals on the snails present in the drainage ditch. They were first located in August, 1933, and were present in large numbers on the moist area at the edge of the water. As the water receded during dry periods it was followed by the snails and when the ditch became completely dry the snails burrowed into the mud where they remained until water was again present. Snails were collected in large numbers from this ditch during the autumn and early winter months but none could be found after December 26, 1933. Observations were continued through January and February, 1934, but no snails were seen before March 3. At this time a single specimen was found after an hour of

searching. One week later 4 specimens were collected. These specimens were all large. By the latter part of March these older specimens were found frequently and very young snails were observed in steadily increasing numbers during the month of April.

The first snails used for experimental purposes were collected during September, 1933, from the previously mentioned drainage ditch which was not accessible to carriers of the adult worms. However, many of the snails were examined for developmental stages but not a single naturally infested snail was found throughout the months in which collections were made from this locality. These snails were placed in aquaria which were prepared to approximate as nearly as possible the natural habitat of the snails. Large and small galvanized tin tubs were filled with dirt taken from the natural habitat and this dirt was banked to one side so that when water was added there was a dry area at the top of the bank, a moist area along the water's edge, and then water which was kept at a fairly constant level. To make the habitat still more natural, grasses, weeds and aquatic vegetation were planted in the aquaria. These aquaria were placed against the southern wall of a large building where they were exposed to the sunlight for the greater part of each day. They remained uncovered except during rains. To supplement the food supply planted in the aquaria, lettuce, cabbage and cauliflower leaves were added when needed. None of the vegetation available to the snails was eaten until it was partially decayed. The snails kept under these conditions seemed to live as well as in their natural habitat. The rate of reproduction was rapid and a constant supply of laboratory raised snails was available after the first few weeks. Under these conditions the snails did not hibernate during the colder months, i.e., January and February, and there was some reproduction although less than in the warmer months. The eggs are usually deposited at the edge of the water but are sometimes found in moist depressions some distance from the water.

The snails were very seldom found in the water or at the dry top of the bank, preferring in the aquaria the same habitat as in nature. Usually when found in water they are attached to some piece of decaying vegetation.

Penetration Experiments.—The snails were infested by placing them singly in small glass dishes containing from 3 to 5 miracidia or by exposing a large number of snails to hundreds of free-swimming miracidia in large containers. The miracidia are attracted to the snails almost immediately, but apparently penetration takes place slowly and only after prolonged exposure of the snails to them. Observations were made many times on the reactions of the miracidia but none was ever observed to

penetrate the snail. In experiments with only a few miracidia in a dish with a single snail they have been observed for as long as 3 hours and at the end of this time all of the miracidia were still present in the dish. Possibly the constant activity of the snail during observation under the microscope and the abnormal conditions under which the miracidia are placed prevented them from entering the snail. The percentages of infestation were always low when the snails were exposed to only a few miracidia, usually about 10% becoming infested. The method of exposing snails to many miracidia resulted in 100% infestation in most cases, but there is danger of over-infestation. However, it was found that the best results could be obtained by exposing the snails in this way for a period of 2 to 4 hours. In earlier experiments snails were left overnight in dishes containing many miracidia, but the subsequent death rate among the snails was so great that only an occasional individual would live long enough to shed cercariae.

The natural inclination of the snails to leave the water adds to the difficulties in securing 100% infestation. To secure the best results in this respect it was found necessary to place in the containers some food material which served to attract the snails into the water. Under these conditions the snails remain relatively motionless, giving the miracidia ample time and opportunity to penetrate them. The miracidia collect at the anterior ends of the snails where they can be seen attaching themselves to the head, foot, and mantle, and at times to the shell. Their attachment usually is very brief, as they are shaken off by slight movements of the snail or they release themselves. They may then attach themselves again at the same point, select another, or swim away, finally becoming attached to another host. At times the snails seem to have no attraction at all for the miracidia. In such cases miracidia were observed swimming in close proximity to several snails but never made any attempt to penetrate them. In view of some of the recent observations on immunity of infested snails this failure of miracidia to penetrate might be due to earlier infestation by this same or other trematode larval forms, but extended observations failed to yield any evidence of natural infestation in the stock of snails used in these experiments. Experiments also demonstrated that these snails do not become immune to infestation by the miracidia of *C. cotylophorum* when previously infested by this same species. Snails containing larval stages as much advanced as mature rediae were exposed to miracidia, and large numbers of the latter penetrated and began development. Miracidia were observed collecting around snail faeces and débris scraped from the bodies of the snails. These substances produced reactions in the miracidia alike in all respects to those produced by the snails themselves. Apparently the attraction was as

strong as that of the snails since the miracidia continued to collect around them even though there were many snails in the container.

The penetration of the miracidia into the snail host was never observed, even though many hours were spent in attempting to do so. However, Krull (1934:176) observed that this miracidium penetrated the head and mantle of *Fossaria modicella* within 15 minutes. In order to determine the point of entrance in the present experiments a few medium-sized snails were exposed to large numbers of miracidia for 6 hours and were then fixed. Upon sectioning and staining these snails many miracidia were found to have penetrated all the exposed surfaces of the snail but principally the mantle and dorsal surface of the foot.

The habitat of *F. parva* and the fact that it feeds on moist decaying vegetation at the edge of the water, where eggs deposited in the faeces of cattle would remain viable, led to experiments to determine whether or not ingested eggs would hatch in the intestine and the miracidia penetrate the intestinal wall. Some small snails were placed in a dish containing eggs almost ready to hatch which were readily eaten by the snails. A few of the snails were fixed immediately after eating the eggs, while the rest were kept for 24 hours before being fixed. It was noticed that some of the eggs were passed in the faeces of the snails unhatched and unharmed since many of them were observed to hatch subsequently. The fixed snails were sectioned and stained, and a careful search was made for miracidia which might have penetrated the wall of the digestive tract at any point, but none was found.

Numerous experiments were made during the fall and winter months of 1933 with very young, medium-sized, and old snails and it was found that the miracidia infested all ages equally well. However, in these experiments large numbers of miracidia were used and as a result most of the snails died before cercariae were shed. The young snails when severely infested usually die within 15 to 20 days while the older ones live until the liver is completely destroyed by developing cercariae. In the young snails which died only sporocysts and young rediae were found.

Relation of Temperature to Development.—One group of snails infested on October 5, 1933, shed cercariae on November 17, after a lapse of 44 days. A second group infested on October 31 shed cercariae on December 23, after 54 days, and a third group infested on December 20 shed cercariae on March 30, 1934, after 91 days. The time required for development increased as the winter months advanced. Late in the spring and in the early summer months of 1934 another series of experiments was performed and in this series the time required for development decreased as the temperature rose. Snails infested on April 25

shed cercariae on June 1 (37 days); a second group infested on April 26 shed cercariae on June 2 (37 days); a third group infested on May 5 shed cercariae on June 5 (31 days); a fourth group infested on May 11 shed cercariae on June 12 (32 days); a fifth group infested on May 15 shed cercariae on June 14 (30 days); and a sixth group infested on June 23 shed cercariae on July 24 (31 days). The results of these experiments are summarized in Table 8.

TABLE 8.—DATA SHOWING TIME REQUIRED FOR DEVELOPMENT OF CERCARIAE OF *Cotylophoron cotylophorum* AT DIFFERENT TIMES OF THE YEAR

No.	Date of infestation	Date on which cercariae were shed	Days elapsed
1.....	Oct. 5, 1933	Nov. 17, 1933	44
2.....	Oct. 31, 1933	Dec. 23, 1933	54
3.....	Dec. 20, 1933	Mar. 30, 1934	91
4.....	Apr. 25, 1934	June 1, 1934	37
5.....	Apr. 26, 1934	June 2, 1934	37
6.....	May 5, 1934	June 5, 1934	32
7.....	May 11, 1934	June 12, 1934	32
8.....	May 15, 1934	June 14, 1934	30
9.....	June 23, 1934	July 24, 1934	31

These data show that the effect of temperature on the rate of development of the stages in the snail host is very comparable to the effect on the rate of development of the miracidium. The time required for the development of the miracidium increased from 11 days in the warmer months to 29 days during the colder months of the year. In the same way the time required for the development of the cercariae increased from 30 to 91 days. Krull (1934:174) found that snails infested with the miracidium of *C. cotylophorum* on May 14 began to shed cercariae on June 19, after a lapse of 36 days. This result agrees very closely with the foregoing since snails infested on May 15 began to shed cercariae on June 14, representing a difference of only 6 days from Krull's data. Krull performed only the one experiment so that no other comparisons can be made. Suzuki (1931:97) performed similar experiments on the development of *Fasciola hepatica* and obtained results comparable to those presented here. He found that a period of 30 days was required for the development of these stages during the summer months of July and August but that from 60 to 70 days were required during the winter months of January and February.

The data secured on the time required for the development of the cercariae during the warmer months indicate that 30 days is approximately the minimum time required. The average time required for de-

velopment of the cercariae during these warmer months was 33 days. The rate of development of the sporocyst, redia, and cercaria was studied in all the experiments shown in Table 8—by dissection of snails at intervals, and also by fixing, sectioning, and staining snails. In this way it was possible to determine at what time the various developmental stages make their appearance. The following discussion is based on the development of these stages in snails infested on May 5. Cercariae were shed by these snails on June 5, after a period of 32 days.

SPOROCYST

DEVELOPMENT

Method of Study.—The earlier stages in development of the sporocyst were studied from sectioned material only. The minuteness of these stages combined with the opacity of the snail shell made accurate observations of living material impossible. To obtain representative stages some of the snails were fixed at 12 hours, and others after 1, 2, 5, 10, 15, 20, 25, 30, and 35 days from the time of exposure to miracidia. In order to supplement the data acquired from sectioned material many snails were dissected after the fifth day of development, at the ends of the given periods and at intervals not indicated, in order to study the living forms.

Sporocyst from Penetration of Miracidium to End of 12 Hours.—As has been stated previously, the miracidia were not observed to penetrate the snail host, but it was possible to determine the points of entrance from sectioned material. The miracidia were never observed to shed the ciliated epidermal cells and evidence from sectioned individuals within the snails clearly demonstrated that the epidermal cells are present after penetration. In several instances miracidia were found in the lymph spaces of the foot and in the body cavity, with all or a part of the cells still attached (Figs. 24, 29). The exact time at which they are lost was not determined but there is no evidence of their presence 12 hours after penetration. The cells are sloughed first from the anterior end of the body as indicated by the fact that many individuals were found on which only the last one or two tiers were present. The opposite condition was never observed. Looss (1892:156) observed that the ciliated cells of the miracidium of *Diplodiscus subclavatus* were retained for 24 hours and until it had reached the liver or ovo-testis. The miracidium of *P. cervi* according to Looss' description (1896:186) does not lose its ciliated cells until after penetration and changes accompanying transformation

into the sporocyst have begun. Takahashi (1928:278) made similar observations on the miracidium of *P. cervi*. Thomas (1883:114; Fig. 7) observed the same phenomenon in the miracidium of *Fasciola hepatica*. He states:

The outer layer of ciliated cells is lost, whilst the embryo changes in form. The ciliated cells absorb water and appear as round or hemispherical vesicles with the cilia standing out perpendicularly from their surface

He does not state how soon after penetration these cells are lost, and it was not determined for the present miracidium. However, they were seen only on miracidia which had undergone the least change at the end of 12 hours after penetration. Doubtless these changes are initiated immediately after the miracidium gains entrance into the snail.

Ameel (1934:289) observed the penetration of the miracidium of *Paragonimus westermanni* but could not determine whether or not these cells are lost although Nakagawa (1917:302) noted that the cells are lost during penetration. Mathias (1925:44; fig. 9a) has described and figured the loss of these cells during the penetration of the miracidium of *Strigea tarda*. Barlow (1925:34; text-fig. 6) made similar observations on the miracidium of *Fasciolopsis buski* as did Ishii (1934: figs. 1, 2, 3) for the same form. Rasin (1933:102) observed that the epidermal cells are shed by the miracidium of *Echinoparyphium recurvatum* before entrance into the snail host. In view of these observations it is only possible to conclude that the ciliated epidermal cells are lost by some miracidia during penetration but are carried into the snail host by others.

The appearance of the miracidium is not greatly altered for some time after penetration, many of the structures being still recognizable. The structures most altered in appearance are the primitive gut and the brain. No contents can be seen in the gut even in miracidia which have just penetrated the snail, as indicated by their very superficial positions. The nuclei of the gut are recognizable in some miracidia but they disappear before the end of the first 12 hours after penetration. The brain degenerates rapidly, not being definitely recognizable in any miracidia after penetration. In a number of miracidia a small round structure was observed, occupying the position of the brain in the free-swimming forms, which might have been the brain in an advanced state of degeneration. This structure measured 12 μ in diameter.

The nuclei of the penetration glands were not seen in miracidia after penetration, but occasionally individuals were seen in which the ducts of the glands were very conspicuous. In some individuals at the end of the first 12 hours the anterior part of the body possesses no recognizable structures other than a few nuclei (Fig. 29).

The appearance of the subepithelial cell nuclei remains the same

through the first stages of transformation but there is a great reduction in number. The central cavity of the miracidium remains and one or two germ balls and many germ cells are present in it. The excretory system is unchanged from that typical of the miracidium.

As transformation takes place the miracidium loses its elongate shape and becomes gradually more ovoid. A very thin cuticula is formed around the outside of the body. Accompanying the change in shape and the loss of miracidial organs is a decided decrease in size. The size of 10 sporocysts which were approximately 12 hours of age is given in Table 9.

TABLE 9.—SHOWING THE SIZE (IN MILLIMETERS) OF 12-HOUR AND 24-HOUR SPOROCYSTS

No.	12 Hours	No.	24 Hours
1.....	0.070 x 0.035	1.....	0.077 x 0.024
2.....	0.054 x 0.032	2.....	0.069 x 0.038
3.....	0.052 x 0.037	3.....	0.069 x 0.033
4.....	0.052 x 0.025	4.....	0.069 x 0.024
5.....	0.048 x 0.032	5.....	0.064 x 0.030
6.....	0.046 x 0.031	6.....	0.061 x 0.032
7.....	0.046 x 0.024	7.....	0.061 x 0.030
8.....	0.045 x 0.034	8.....	0.061 x 0.026
9.....	0.045 x 0.029	9.....	0.061 x 0.023
10.....	0.039 x 0.032	10.....	0.060 x 0.018
Average.....	0.050 x 0.031	Average.....	0.065 x 0.028

Barlow (1925:34) observed that the sporocysts of *Fasciolopsis buski* never became immobile and that the digestive tract became larger and functionally more active as the sporocysts grew in size and that they could be observed feeding at all times. Before rediae were born he found that the sporocysts had migrated well into the body of the snail. These observations could not be confirmed in the present material. The positions in which the sporocysts develop indicate that some of the miracidia either swim in the body fluids or are passively carried by movements of the liquids in the cavities of the snail's body. However, they become permanently located soon after penetration. In some instances young sporocysts were found free in the body spaces surrounding the radula and esophagus, and subsequent findings indicate that sporocysts develop attached to the walls of this cavity. Other young sporocysts were observed in all parts of the foot, including the center of this muscle mass, which further demonstrates that some movement from the point of penetration does occur. The sporocysts apparently prefer the mantle tissues to any other tissues of the snail's body since more of them develop in this region than elsewhere in the body.

24-Hour Sporocyst.—The initial rate of development is very slow when the size of the sporocyst is taken as a criterion. Perhaps this is due to the fact that an almost complete transformation occurs and to the fact that the sporocyst must become established before it receives adequate nourishment to provide for rapid growth. There are two notable changes which occur by the end of the first 24 hours in the snail. The first is the initiation of growth, as shown in Table 9, and the second is the breaking down of the germ balls which were present in the miracidium (Fig. 28). The germ cells, separated from each other by the breaking down of the germ balls, are scattered in the central cavity, almost occluding it. No cell boundaries could be distinguished, thus giving the body the appearance of a syncytium. The germ cell nuclei measure from 4 to 6 μ in diameter and have lost the appearance characteristic of these nuclei in the miracidium. The chromatin is no longer concentrated in one central body surrounded by distinct masses but is uniformly scattered throughout the nucleus. Suzuki (1931: figs. 12, 13) has figured a similar stage in the young sporocyst of *Fasciola hepatica*. Mathias' (1925:44) description of this stage of development of the sporocyst of *Strigea tarda* is not complete but apparently it is very similar to that of the present material. Brooks (1930:302; fig. 1) has described and figured these scattered germ cells in the young sporocyst of *Cercaria lintoni* Miller which he designates as "antecedent germ cells."

Several elongate nuclei which measure 4 by 2 μ were observed near the periphery of the body. These are probably subepithelial nuclei. The anterior end of the body at this age appears as a translucent, granular mass in which remains of miracidial structures can be seen occasionally.

48-Hour Sporocyst.—After two days in the snail host the sporocyst no longer has any trace of the miracidial structures, although the anterior end of the body is still filled with a translucent tissue in which only an occasional nucleus is located. There is a decided increase in size over the 24-hour stage (Table 9). The most important development is that of the embryonic rediae. The origin of these was not established but it is possible that they are derived from the germ cells liberated by the breaking down of the miracidial germ balls, the "antecedent germ cells" of Brooks. The "germ mass" and "components" described by Brooks as being derived from the "antecedent germ cells" in the sporocyst of *C. lintoni* were not observed in the sporocyst of *C. cotylophorum*. The fact that the structures seen in the 48-hour sporocyst are embryonic rediae and not "germ masses" is clearly demonstrated by their subsequent development.

The size and number of young rediae present in 48-hour sporocysts is shown in Table 10. At a very early age these embryos have a definite

TABLE 10.—SHOWING THE SIZE (IN MILLIMETERS) OF 48-HOUR, AND 5-, 10-, AND 15-DAY SPOROCYSTS, THE NUMBER OF REDIAE IN EACH, AND THE SIZE OF THE LARGEST REDIA IN EACH

Age and No.	Sporocyst	Number of rediae	Largest redia
48 hours:			
1.....	0.070 x 0.038	1	0.023 x 0.016
2.....	0.070 x 0.053	1	0.015 x 0.015
3.....	0.077 x 0.033	1	0.023 x 0.015
4.....	0.082 x 0.043	2	0.025 x 0.018
5.....	0.092 x 0.046	1	0.018 x 0.018
6.....	0.100 x 0.046	3	0.021 x 0.015
7.....	0.101 x 0.046	2	0.023 x 0.018
8.....	0.107 x 0.053	3	0.021 x 0.020
9.....	0.123 x 0.026	2	0.023 x 0.015
10.....	0.123 x 0.028	2	0.027 x 0.018
Average.....	0.095 x 0.041	..	0.022 x 0.017
5 days:			
1.....	0.126 x 0.084	3	0.042 x 0.042
2.....	0.138 x 0.061	3	0.053 x 0.043
3.....	0.161 x 0.053	3	0.046 x 0.046
4.....	0.168 x 0.097	4	0.063 x 0.050
5.....	0.168 x 0.105	5	0.067 x 0.054
6.....	0.170 x 0.078	4	0.052 x 0.052
7.....	0.170 x 0.110	5	0.069 x 0.053
8.....	0.200 x 0.086	4	0.058 x 0.058
9.....	0.226 x 0.084	5	0.073 x 0.047
10.....	0.218 x 0.092	5	0.080 x 0.063
Average.....	0.181 x 0.085	..	0.060 x 0.051
10 days:			
1.....	0.160 x 0.080	5	0.076 x 0.029
2.....	0.218 x 0.063	5	0.105 x 0.038
3.....	0.260 x 0.134	5	0.105 x 0.055
4.....	0.268 x 0.151	5	0.139 x 0.092
5.....	0.294 x 0.105	8	0.151 x 0.046
6.....	0.302 x 0.100	5	0.134 x 0.088
7.....	0.336 x 0.134	5	0.126 x 0.084
8.....	0.395 x 0.189	5	0.168 x 0.050
9.....	0.420 x 0.168	7	0.189 x 0.088
10.....	0.433 x 0.160	9	0.189 x 0.046
Average.....	0.309 x 0.126	..	0.138 x 0.062
15 days:			
1.....	0.273 x 0.189	5	0.168 x 0.050
2.....	0.294 x 0.160	6	0.197 x 0.055
3.....	0.315 x 0.156	5	0.216 x 0.061
4.....	0.315 x 0.210	6	0.193 x 0.046
5.....	0.320 x 0.210	5	0.155 x 0.080
6.....	0.323 x 0.151	7	0.210 x 0.050
7.....	0.370 x 0.181	8	0.168 x 0.063
8.....	0.376 x 0.134	8	0.189 x 0.055
9.....	0.420 x 0.189	9	0.189 x 0.063
10.....	0.470 x 0.285	8	0.225 x 0.080
Average.....	0.348 x 0.186	..	0.191 x 0.060

shape and each is enclosed in a firm membrane which Dubois (1928:63) designates as a primitive epithelium (Fig. 31). In addition to the definitely formed rediae there are many germ cells present in the posterior part of the body. These cells measure 8 to 10 μ in diameter and have large nuclei which measure 6 to 7 μ in diameter. The cytoplasm of these cells stains more darkly than the surrounding tissues in which cell boundaries are not distinguishable. The chromatin of the nuclei is arranged in one large body eccentrically placed and several small masses. Cleavage of these cells is unequal.

Some of the 48-hour sporocysts show a definite central cavity but in others no cavity could be seen. The cuticula around the outside of the body is considerably thicker than in the 24-hour sporocyst.

5-Day Sporocyst.—Between the second and fifth days the sporocyst and enclosed rediae increase very rapidly in size (Table 10). The central cavity becomes more distinct and the body walls become much thinner. At the two extremities the body is filled by a large number of cells, making the walls much thicker in these regions than they are laterally. In sectioned specimens the difference in thickness of these regions is not so evident as in living specimens. This is due to the fact that the developing rediae are uniformly placed in an undisturbed sporocyst and keep it more extended than in a sporocyst dissected from a snail. When fixed quickly the sporocysts do not contract, but living specimens usually contract at both extremities, forcing the rediae to the center of the body (Fig. 25), and consequently the two extremities appear to be very thick-walled. The number of rediae in 5-day sporocysts is variable but is never found to exceed 5. The size of the largest redia in each of 10 sporocysts of this age is shown in Table 10. No structures of the redia are recognizable at this stage in development.

10-Day Sporocyst.—A very few of the sporocysts in this experiment reached their maximum size in 9 days and rediae were found free in the tissues of the snail. The size of 10 sporocysts and the largest redia in each is shown in Table 10. The time required for the sporocyst to reach this stage of development during the winter months was very much longer. In the experiment begun on December 20, 1933, no free rediae were found in the snails until February 2, 1934, representing a difference of 35 days required to reach similar stages.

15-Day Sporocyst.—By the fifteenth day many rediae were found free in the snail, but as indicated in Table 10 only a small number of the sporocysts had reached their maximum size.

An occasional sporocyst was found in snails as long as 35 days after infestation, which in this experiment was after cercariae were being shed.

These sporocysts were usually located in the foot where conditions were perhaps not as favorable for growth as in other parts of the body. Others were found in the anterior margin of the mantle. Many authors have reported extensive migrations by the sporocysts of other species of trematodes, but the sporocysts of the present species were never found posterior to the anterior margin of the kidney. In most instances the sporocysts were found completely surrounded by an unbroken layer of cells produced by the snail, which indicates the relative immobility of the sporocyst.

The number of fully developed and developing rediae was never found to exceed 9 in a mature sporocyst (Fig. 35). Usually there are one or two ready to be liberated, while the remainder are in various stages of development. There is no birth pore in the sporocyst and the rediae can be liberated only by rupturing the body wall. The rediae most advanced in development are always located at the anterior end of the sporocyst, and it is this region of the body which is ruptured. A number of sporocysts were observed in which this rupture was evident. It was always at the extreme anterior end, and posterior to it the sporocyst was strongly contracted, causing the torn end to flare out. The constriction of the body prevents the less developed rediae from escaping. Thomas (1883:120) states that this constriction is maintained until the rupture is healed but this observation could not be confirmed. It is probable that the act of rupturing the body wall is initiated by the rediae, but observations made on sporocysts containing advanced rediae indicate that the sporocyst is an active participant in the process. When a redia is ready to emerge it moves or is forced into the anterior end by contraction of the body wall. The sporocyst then contracts behind it forcing it strongly against the anterior end of the body, and in this way apparently takes a part in rupturing its own body wall (Fig. 36).

Old sporocysts which have ruptured walls contain only a few rediae, some having been observed in which there were only 3 present. This fact, combined with the fact that they decrease rapidly in number in infested snails, points to the conclusion that each sporocyst will produce a definite number of rediae. For this species the number is probably 9.

Excretory System.—The excretory system of the sporocyst consists of the two original flame cells and their ducts present in the miracidium. The flame cells are readily visible at all times in the living specimens and it is quite easy to trace the ducts. These structures increase in size as the sporocyst develops, the flame cells reaching a size of 15 by 5 μ . The course of the ducts in the young sporocyst is exactly the same as in the miracidium (Fig. 25) but tends to become straighter as the sporocyst

becomes larger (Fig. 26). The course followed by these ducts depends quite naturally on the state of contraction of the individual and in some contracted old sporocysts the convolutions performed by them are very similar to those in younger specimens. The small bladder present at the end of each duct in the miracidium is also present in the sporocyst.

Shape.—The shape of the sporocyst as seen in sectioned material is ovoid at all stages of development. When young specimens are dissected out of the host into physiological salt solution they are capable of assuming a spherical shape, but the older specimens cannot contract to this extent, due to the presence of large rediae. They are able to contract the extremities strongly, and freed specimens usually have the posterior end more strongly contracted than the anterior end.

Muscles and Activity.—The circular muscles are strongly developed throughout the body, as evidenced by their activity, but the longitudinal ones seem to be less well developed. Very young specimens when freed from the host assume the spherical shape immediately, and subsequent movements are so slight as to be hardly noticeable. The fully developed sporocysts are relatively active. Their activity consists of slow contractions of the muscle layers which are too weak to produce any appreciable progression.

Appearance.—The fully developed sporocyst is visible to the unaided eye but cannot be readily distinguished from rediae or from small particles present in the water. The outside of the body is covered by a mucus which is evidenced by the ability of the sporocyst to cling to the bottom of a dish or to a slide and by the amount of débris which adheres to it. The cuticula on the outside of the body is thrown into fine transverse striations by the contraction of the longitudinal muscles, which gives the body a distinctly ridged appearance. The simultaneous contraction of the muscle layers at the anterior end of the body produces a knobbed appearance (Fig. 25). This condition is so characteristic of the living sporocyst that one is able to distinguish it from rediae and to orient it very quickly with the aid of the microscope.

MATURE SPOROCYST

The mature sporocyst is elongate, usually bluntly rounded at the two extremities, and circular in cross section. It is relatively simple in structure, consisting of a wall of variable thickness surrounding a central cavity (Fig. 30). The walls are composed of a thin cuticula, a layer of circular and longitudinal muscle, and an epithelial layer.

The cuticula is from 2 to 3 μ thick when measured in mature sectioned sporocysts, but when measured on living specimens of the same

age it appears to be from 5 to 6 μ thick, as seen in optical sections. This difference is due partially to the contracted state of the living specimens and partially to the fact that less accurate measurements can be made on living material. Immediately beneath this layer are the circular muscles, which can be seen distinctly. The longitudinal muscles were not seen, although they were looked for with a magnification of approximately 1500 diameters. The thickness of the combined layers is only 3 μ .

The epithelial layer consists of large vacuolated cells and small cells with a granular, deeply-staining cytoplasm (Figs. 27, 30). The large cells are more numerous than the others and comprise most of the body wall. They measure from 30 to 40 μ by 20 to 35 μ and contain nuclei which measure from 8 to 10 μ . A distinct chromatin mass, usually eccentric in position, is present in all of them. There are also many granular masses of chromatin scattered throughout the nuclei. The small cells measure approximately 18 by 9 μ and the diameter of the nuclei is from 6 to 7 μ . The chromatin of these nuclei has the same arrangement as that in the nuclei of the larger cells but is more dense. These small cells are readily distinguished from the larger cells by the difference in size and by their darker staining reaction. The distribution of these cells is very irregular. They may be located in contact with the muscle layer or scattered among the larger cells, although more of them were found in the posterior tip of the body than elsewhere.

That these small cells are probably germinal is indicated by their location and similarity in size and staining reaction to the cells of very young rediae (Fig. 30). Many workers have described similar cells in sporocysts of other species as germ cells.

Thomas (1883:115) in his description of the life cycle of *Fasciola hepatica* says:

The contents of the sporocyst are formed by a number of very clear rounded cells, some of which are the germinal cells of the embryo or cells derived from them by division, others are formed by a proliferation of the epithelium lining the cavity of the sporocyst.

Looss (1896:187) states of the sporocyst of *Paramphistomum cervi*:

Tandis que sur la paroi interne du sporocyst, on ne rencontre que rarement, . . . des cellules germinatives normales, celles-ci se présentent amassées dans l'extrémité caudale où elles vont former un véritable épithélium germinatif.

Mathias (1925:50) describes two kinds of cells in the walls of the sporocyst of *Strigea tarda* which are similar to those present in the wall of the present sporocyst. The smaller of these he believes to be germ cells. Dubois (1928:63) describes similar cells irregularly dispersed in the body wall of the sporocyst of *Cercaria helvetica* v which he considers to be germ cells. Brooks (1930) in his detailed study of the germ cell cycle in 20 species of trematodes did not find any evidence to support the

theory that any cells in the epithelial layer of the sporocyst wall were germ cells. Price (1931:709) believes that the larger of the two types of cells found in the sporocyst wall of *Schistosomatium douthitti* are germ cells.

It is not my purpose to enter into the merits of the many conflicting viewpoints, but in the present material only 9 rediae are produced in each sporocyst, and I believe that the germ cells producing these are formed in the germinal tissue of the miracidium prior to its penetration into the snail host. If the small, deeply-staining cells present in the wall of the sporocyst are germ cells then the vast majority of them never produce rediae.

The rediae develop entirely enclosed by sporocyst tissue which divides the cavity of the sporocyst into compartments and in which the rediae remain until late in development. The fibers seen attached to developing rediae by Looss (1892:159) in the sporocyst of *Diplodiscus subclavatus* and by Price (1931:709) in the sporocyst of *Schistosomatium douthitti* are probably the same structures described here.

REDIA

Rediae belonging to the family Paramphistomidae have been described by a number of authors. Looss (1892) studied the development of the redia of *Diplodiscus subclavatus* and described the developmental stages and the mature redia in great detail. He also (1896) described briefly the rediae of *Paramphistomum cervi* and *Gastrodiscus aegyptiacus*. Cort (1915) gave a few details concerning the structure of the rediae of *Cercaria inhabilis* and *C. diastropa*. Faust (1919, 1919a) did the same for the rediae of *Cercaria frondosa* and *C. convoluta*. Sewell (1922) described rather completely the rediae of *Cercariae Indicae* xxi, xxvi, xxix, xxxii. McCoy (1929) gave a brief description of the redia of *Cercaria missouriensis*. Beaver (1929) described the redia of *Allasosotoma parvum*. Le Roux (1930) mentioned the fact that daughter rediae occur in the life cycle of *Cotylophoron cotylophorum* but gave no morphological details. Krull and Price (1932) described very briefly the redia of *Diplodiscus temperatus*.

These rediae belong to the subfamilies Paramphistominae Fiscoeder 1901 and Diplodiscinae Cohn 1904. The rediae of *P. cervi*, *C. Indicae* xxvi, xxix, xxxii, and *C. cotylophorum* belong to the subfamily Paramphistominae. These rediae are readily distinguished from those of the Diplodiscinae by the absence of lateral appendages. In general, the rediae of the Paramphistominae are smaller and possess a smaller pharynx and gut, although these characteristics are not of diagnostic value.

DEVELOPMENT

Structure of the Redia.—The redia is much more complex in structure than the sporocyst. It possesses a well developed digestive tract consisting of a mouth, pharynx, esophagus, rhabdocoel intestine, and a large number of unicellular glands which are associated with it. The redia also possesses a more complex excretory system, a discernible central nervous system, and a birth pore.

Rate of development.—Development of the redia in the sporocyst is very rapid and at the time of liberation all the structures of the mature redia are present, with the exception of the birth pore. In the experiment under discussion the first rediae free in the body of the snail host were found 9 days after infestation.

The growth of the redia in the sporocyst was studied in an attempt to establish the chronological sequence of organ development and to determine the time of germ ball development. During the first 3 days the redia is spherical in shape and consists of numerous cells with indistinct boundaries which contain nuclei varying from 3 to 6 μ in diameter. On the fourth or fifth day the redia begins to elongate and the primordia of the digestive system appear. The smallest embryo with the primordia measured 68 by 45 μ (Fig. 42).

Digestive System.—The primordia of the digestive system consist of a group of centrally located cells whose cytoplasm is finely granular. These cells measure 10 to 12 μ in diameter and contain relatively large nuclei, 6 to 7 μ in diameter. Looss (1892:160) states that these cells produce a secretion which forces them apart, thus producing the lumen of the digestive tract. The cause of the separation was not determined in the present material, but the lumen is produced by a delamination of the primordial cells. As the embryo continues to grow the digestive primordia increase rapidly in size, extending from near the anterior end almost to the posterior end in embryos measuring 80 to 50 μ . In rediae of this size a small number of loosely organized cells which are destined to form the pharynx are present at the anterior end of the digestive tract. The lumen of the intestine becomes much more evident at this stage, being widest at the middle of its length. Anteriorly it is much narrower where it joins the pharyngeal cells.

Following this stage it rapidly assumes the appearance characteristic of it in the mature redia. The pharynx becomes definite in shape, a basal membrane develops around it and the intestine, and muscle fibers begin to develop in the pharynx. The development of muscles in the pharynx is accompanied by a breaking down of the cell membranes of the cells from which it develops. However, the nuclei of these cells remain dis-

tributed irregularly through it. In an embryo 106 by 55 μ , representing this stage in development, the pharynx measured 26 μ wide and 16 μ long and was located 14 μ from the anterior end of the body. The intestine does not increase in length to accompany the increase of body length. In the above specimen it terminated 27 μ from the posterior end.

Early in development, when the embryos have reached a length of approximately 90 μ , the cuticula lining the mouth cavity, pharynx, and upper part of the esophagus begins to form. Six cells at the anterior end of the intestine, which are designated as pharyngeal cuticula cells, grow forward through the lumen of the pharynx but do not entirely occlude it. The cells are united at their anterior ends, forming a cap which closes over the lumen (Fig. 44). As growth continues the cells elongate and their anterior ends approach the surface of the body. At this stage the cells measure 31 μ in length and 6 μ in width at the posterior end. At the anterior ends of these cells and near the surface the primitive epithelium covering the body grows inward around them until it reaches the anterior margin of the pharynx, where it seems to fuse with the inner surfaces of the cells in embryos approximately 100 μ in length. That this process is a result of growth and not of invagination of the anterior part of the body was demonstrated by study of serial sections of the embryo. The primitive epithelium was found to be intact over the entire surface of the body. That portion of the body lying directly anterior to the pharynx, represented by the nuclei in Fig. 44, is eventually sloughed and apparently forms a plug which fills the mouth cavity in slightly larger individuals (Fig. 49). The function of this plug is unknown.

At the time of the fusion of the primitive epithelium with the pharyngeal cuticula cells a substance is deposited in the surface cells next to the lumen of the pharynx. This substance, which forms the cuticular lining of a part of the mouth cavity, the pharynx, and a part of the esophagus, stains darkly in haematoxylin and is very difficult to destain. This characteristic indicates its extent in older forms, since the cuticula produced by the primitive epithelium does not stain so deeply. Accompanying the formation of the pharyngeal cuticula the nuclei of the cells producing it degenerate. The nuclei first become flat and elongate, the chromatin then forms a large central mass, and finally the nuclei disappear entirely, leaving a uniformly thin cuticula continuous with that of the body. The nuclei of the primitive epithelium degenerate also during its transformation into the cuticular covering of the body, which is concurrent with the formation of the pharyngeal cuticula.

Simultaneously with the formation of the pharyngeal cuticula, 6 other cells, which are designated as esophageal cells, become differentiated at

the anterior end of the intestine. These cells are broad at the base but become thin anteriorly at their junction with the pharyngeal cuticula cells (Fig. 43). The esophageal cells either produce a cuticula-like substance or are covered externally by the secretions of the pharyngeal cuticula cells, since they stain in a similar manner during the early stages of their development. Later this staining reaction disappears. However, the fact that this is cuticula is proved by the stiffened esophagus which projects prominently into the lumen of the intestine in contracted rediae.

In embryos 150 μ in length the formation of these structures is complete, the mouth is open but still contains the plug, and the entire embryo is still enclosed by sporocyst tissue (Fig. 40). The pharynx is 34 μ wide and 22 μ long, and the intestine, which is approximately 40 by 30 μ in embryos of this size, extends slightly past the middle of the body. The intestine consists of a single layer of distinct cells which measure 10 to 15 μ in diameter. Each contains a relatively large nucleus. The basal membrane which encloses both the pharynx and the intestine contains distinct ovoid nuclei early in development but they could not be found in the membrane in rediae of this size. Looss' (1892:161) observation that muscles develop in this membrane was not confirmed for this redia.

No rediae larger than 150 by 52 μ were found enclosed in sporocyst tissue, nor any which contained the plug in the mouth cavity. Apparently they break out of their individual compartments shortly after reaching this size and the mouth opens.

Looss' (1892:160-161; figs. 6, 7) description and figures of the development of the digestive organs in the redia of *D. subclavatus* show it to be very similar to that of the present redia. He did not observe a sloughing of the primary cuticula, which is produced by the primitive epithelium and pharyngeal cuticula cells, in the redia of his material, but he did observe indications of such a process in the redia of *Cercaria cystophora* (1892:161) after being born. He believes that a similar process occurs in the redia of *D. subclavatus* and that a secondary cuticula is produced by the underlying layers of the body wall. According to his observations neither the mouth nor the birth pore are open until after birth. In *C. cotylophorum* the mouth of the redia is open at least two days before birth but there is no indication of a birth pore. At this stage of development no indications of sloughing of the primary cuticula other than that previously mentioned were observed in the present material at any stage of development. Since no subsequent sloughing was observed it is believed that the primary cuticula is not sloughed at any time and that it forms the cuticula of the mature redia.

Germ Cells.—The germ cells appear simultaneously with the primordia

of the digestive system, that is in individuals approximately $60\ \mu$ in length. At this size living rediae appear as a homogeneous mass of cells surrounded by the primitive epithelium. However, in sectioned material the germ cells are obvious because of their size, position, and staining reaction. They were always found in the posterior region of the body, posterior or lateral to the digestive primordia (Fig. 42). The cells do not have definite boundaries, and the cytoplasm, which is finely granular, takes a deeper stain than the cells of surrounding tissue. They measure approximately 10 by $8\ \mu$ and contain nuclei which are $6\ \mu$ in diameter. There is no distinct central cavity in the young individuals but as the germ cells divide to form germ balls a small space becomes evident around each one. The smallest redia in which a definitely formed germ ball was found measured 85 by $39\ \mu$. In an individual measuring 152 by $54\ \mu$ 4 germ balls were present. Usually there are 10 to 12 present when the redia is born. Early in the formation of the germ balls an occasional ovoid nucleus is observed near the periphery. These are the nuclei of the cells which are destined to form the primitive epithelium around the germ ball. These nuclei were observed in germ balls as small as $15\ \mu$ in diameter, but no definite retaining membrane is formed until they have reached approximately $30\ \mu$ in diameter. The retention of the germ balls in individual compartments is not very evident in the young rediae because of the thickness and irregularity of the body wall but this arrangement becomes very distinct in older specimens (Fig. 39).

Excretory System.—The earliest stages in the development of the excretory system were not observed. Looss (1892:161) states that in *Distomum ovocaudatum* he observed the excretory system of the redia in the 2-flame-cell stage; each cell opened separately at the posterior end of the body. In the present material no stage as early as this was observed. However, I believe that the excretory system becomes functional when the redia is approximately $75\ \mu$ in length. Small lateral tubules toward the posterior extremity were observed in embryos of this size but no flame cells could be distinguished. The smallest redia in which flame cells were observed was 105 by $84\ \mu$. In this individual there were 2 on each side. The anterior pair is located lateral to the anterior end of the intestine, and the posterior pair is located in the extreme posterior end (Fig. 35). The excretory ducts on each side unite to form a common duct which expands to form a small bladder on either side shortly before reaching the excretory pore. In individuals of this size the excretory pore is located approximately one-third of the body length from the posterior end. A third flame cell is developed on each side when the redia is about $160\ \mu$ long, although specimens $135\ \mu$ long which possessed the

third cell were found occasionally. This cell develops near the excretory pore and its duct unites with the duct from the anterior flame cell (Fig. 46).

Nervous System.—The nervous system of the redia develops at the same time as the digestive and excretory systems. The central fibrous nerve mass is located dorsal to the esophagus, and the nerve cells completely surround it (Fig. 37). The region of the body in which it develops is filled by numerous cells at all stages of development and it is very difficult to determine when the nerve cells become differentiated. However, in rediae 100 μ long some nuclei have the appearance which is characteristic of the nerve cell nuclei in older forms. The nuclei are round, measure 4 to 5 μ in diameter, and contain numerous granular masses of chromatin which cause them to stain more darkly than other surrounding nuclei. The nuclei are scarce over the dorsal surface of the fibrous mass but are very numerous lateral to it. They extend to the ventral side of the redia and across the body ventral to the esophagus. The central fibrous mass is approximately 30 by 15 μ in rediae 150 μ in length. No nerves were observed to leave this central mass.

Salivary Glands.—The salivary glands which surround the anterior part of the digestive tract become differentiated in rediae slightly over 100 μ in length, being first observed in an individual which measured 104 by 52 μ . In this redia 6 cells were found which were considered to be gland cells. These glands are characterized by finely granular, deeply-staining cytoplasm and a relatively large nucleus which contains a few granular masses of chromatin (Fig. 34). There is also a concentration of chromatin at the nuclear membrane, while the remainder of the nucleus remains comparatively clear. The cells are drop-shaped with a long slender projection extending anteriorly. The cells are approximately 6 μ in diameter at their posterior ends, and the nucleus, which is located here, is 7 by 4 μ . The anterior extensions of the glands lengthen as the redia grows but do not reach the surface until the mouth cavity is being formed. In a redia about 150 μ in length 40 of these glands were found. Twelve of them were located around the pharynx, some lying anterior to it, while the remaining 28 were distributed around the esophagus and anterior part of the intestine. Looss (1892:161), Cort (1915: 23, 25), Sewell (1922:71, 77, 86), and Krull and Price (1932:9) have described very similar glands in other amphistome rediae.

Muscle Tissue.—The development of the muscular tissues is seemingly very slow. No movement is noticeable in rediae under 125 μ in length. At this size movement consists of very slow and weak contractions of the circular and longitudinal muscles. The individual muscle

layers could not be distinguished in rediae of this size, but the thickness of the combined layers is only 1 to 1.5 μ .

Body Wall.—The body wall in the young redia is similar to that of the young sporocyst. It consists of a very thin cuticula 1 μ thick, the muscle layer, and an inner epithelial layer which is from 10 to 15 μ thick in rediae developing in the sporocyst. The germinal tissue is located in the posterior extremity of the body, where it forms a mass which is approximately 30 μ thick.

Redia Prior to Liberation.—By the end of the seventh or eighth day after infestation the largest rediae are approximately 150 μ long and are similar in every way to mature rediae, except for their lack of a birth pore. The chronological sequence of organ development and germ ball formation is: (1) primitive epithelium, (2) digestive primordia, (3) germ cells, (4) excretory system, (5) nervous system, (6) gland cells, and (7) muscle development, as evidenced by movement. The sequence of organ development and the formation of early germ balls is very similar to that of the miracidium.

Liberation of Rediae—Rediae are found free in the central cavity of the sporocyst for one or two days before they break out into the body of the snail. During these days the only noticeable changes are an increase in the size and number of germ balls. Usually there are only one or two of these more advanced rediae present in the sporocyst at one time. The process of breaking out of the sporocyst was considered in the discussion of the sporocyst development.

The fact that the rate of development is unequal may explain why rediae of variable sizes are liberated by the sporocysts. Some rediae were found free in the snail host at a size of 169 by 52 μ , while on the other hand rediae as large as 225 by 58 μ were observed still in the sporocyst. The average size of 10 at the time of liberation was 188 by 56 μ . Looss (1892:162) found that the rediae of *Diplodiscus subclavatus* were freed when approximately 200 μ in length. At the time of their liberation the rediae contain from 10 to 12 germ balls, the largest of which are approximately 20 by 20 μ . In an individual which measured 225 by 67 μ the pharynx was 29 μ wide by 22 μ in length; the intestine was 71 μ long by 54 μ wide and terminated near the middle of the body length.

Birth Pore.—The rediae begin feeding upon the host tissues immediately after their release and migrate slowly into the liver and ovo-testis where they complete their development. The birth pore is the only structure which develops after they leave the sporocyst. It was first observed in a living specimen which measured 0.27 by 0.1 mm. In contracted specimens it appears as a small projection on the ventral surface of the

body, approximately $160\ \mu$ from the anterior end. Sewell (1922:71) found the birth pore of the redia of *Cercariae Indicae* xxvi to be situated to one side just behind the level of the pharynx, and in the rediae of *C. Indicae* xxix he (p. 77) found it to be ventro-lateral one-fourth of the body length from the anterior end. Beaver (1929:16) found that the birth pore of the redia of *Allassostoma parvum* was dorsal to the anterior part of the intestine, and that it was visible only when cercariae were emerging through it. The central nerve mass of the redia is considered to be dorsal, and the flame cells, excretory ducts, and pore are considered to be lateral. By using the position of the excretory organs for orientation of the redia the birth pore is found to be ventral. In living specimens it can be seen only in a lateral view, and in sectioned material it is found ventral to the brain mass (Fig. 38). The time at which it opens was not determined.

Increase in Size.—The increase in size of the redia is very rapid. In the present experiment the largest redia ever found was dissected from a snail 10 days after infestation (Fig. 48). This specimen was 1.02 by 0.21 mm in size, the pharynx measured 55 by $55\ \mu$, and the intestine was 160 by $84\ \mu$. It contained 23 well-formed germ balls or cercariae, and others seemed to be developing at the posterior end of the body. The largest of the cercariae measured 90 by $65\ \mu$. This individual was perhaps an aberrant form since the next largest specimen ever found measured 0.84 by 0.18 mm and contained only 14 cercariae. Apparently the redia reach their maximum size immediately before the birth of the first cercariae and then decrease somewhat in size as the cercariae are born. The redia in Fig. 33 was taken from the snail mentioned above and was the largest of the remaining rediae. It measured 0.55 by 0.12 mm, the pharynx was 48 by $50\ \mu$, and the intestine was 105 by $64\ \mu$. It contained 15 cercariae. The size of the 10 largest rediae found, the size of the pharynx, the position of the birth pore, and the number of cercariae developing in each is given in Table 11. Many rediae in all stages of development were constantly found in the snails used in this experiment, due to the fact that the sporocysts continued to produce rediae until the termination of the experiment, 35 days after infestation of the snails.

MATURE REDIA

There is no marked change in the appearance of the mature rediae from that of those just born. The pharynx and intestine increase in size but are much smaller in relation to body size than in young rediae. The pharynx of 10 rediae just liberated from the sporocyst averaged 32 by $27\ \mu$, while the average size of this structure in the rediae, given in

Table 11, is 49 by 40 μ . The pharynx was never observed to become elongate, its greatest diameter always being the transverse one. The intestine, which reaches the middle of the body in young specimens, does not extend posteriorly more than one-fifth of the body length in mature forms.

TABLE 11.—MEASUREMENTS (IN MILLIMETERS) OF TEN MATURE REDIAE AND THE NUMBER OF CERCARIAE IN EACH

No.	Size of redia	Size of pharynx	Distance of birth pore from anterior end	Number of cercariae in each
1.....	1.01 x 0.22	0.055 x 0.050	0.180	23
2.....	0.94 x 0.18	0.050 x 0.048	0.174	14
3.....	0.77 x 0.21	0.050 x 0.042	0.165	18
4.....	0.67 x 0.13	0.046 x 0.046	0.147	12
5.....	0.62 x 0.12	0.046 x 0.034	0.189	10
6.....	0.60 x 0.17	0.042 x 0.042	0.178	12
7.....	0.59 x 0.17	0.046 x 0.042	0.162	15
8.....	0.58 x 0.15	0.046 x 0.046	0.155	14
9.....	0.57 x 0.17	0.046 x 0.046	0.170	16
10.....	0.55 x 0.12	0.048 x 0.040	0.148	15
Average.....	0.59 x 0.16	0.049 x 0.040	0.168	15

The excretory system is typical of all the amphistome rediae which have been described, with the exception of the redia of *Paramphistomum cervi* and *Diplodiscus subclavatus*. Looss (1892:161: fig. 10) found 3 or 4 pairs of flame cells in the redia of *D. subclavatus*, and in the mature redia of *P. cervi* there are 5 pairs of flame cells (1896:188). The flame cells in the mature redia of *C. cotylophorum* are located as in the young forms. However, the excretory pore is situated very near the middle of the body length, because of the posterior extension of the body caused by the developing cercariae (Fig. 46). The flame cells are approximately 12 by 5 μ and the ducts, which are only a little coiled, are 2 to 3 μ in diameter. The ducts from the anterior and posterior cells on each side unite slightly anterior to the middle of the body. The duct from the middle cell joins the duct from the anterior flame cell. The common duct formed by the union of the anterior and posterior ducts is approximately 50 by 10 μ in size. It expands to form a small bladder 20 by 30 μ which opens to the outside through a very small canal. The excretory pore when expanded measures 10 μ in diameter. The nervous system remains unchanged in the mature redia. The salivary glands increase in size as the redia grows. In a redia which was 0.35 mm long the cells measured 14 by 10 μ , being very conspicuous in sectioned material

because of this increase in size and a darker staining reaction. In old specimens which have passed maturity these cells stain lighter in haematoxylin stains and seem to be fewer in number.

The germinal tissue, which is located in the posterior extremity of the body, becomes exhausted in the older forms (Fig. 41). This exhaustion was found to occur in some rediae in this experiment 25 days after infestation of the snail, although there were many developing cercariae still present in them. This indicates that the number of cercariae produced by a single redia is very limited. In Table 11 the number present in mature rediae is shown to vary from 10 to 23, with an average of 15. This average doubtless is less than the number produced by each individual which is believed to be nearer 25. If this estimate is approximately correct, then the number of cercariae produced by each miracidium is 225, since each sporocyst produces 9 rediae. Naturally this estimate precludes the formation of daughter rediae. Takahashi (1928: 278) found that the sporocyst of *Paramphistomum cervi* produces 9 rediae, each of which gives rise to 20 cercariae, resulting in a total of 180 cercariae produced by a single sporocyst. Krull (1934:174) attempted to correlate his findings with those of Takahashi based on a total number of cercariae shed by each snail. He found that the average production of each of 11 snails was 152 cercariae; but since he allowed uncounted numbers of miracidia to infest his snails, no accurate estimate could be made as to the number of cercariae produced by a single miracidium. Consequently, he has no basis for his attempt at correlation with Takahashi's observations.

The body wall of the mature redia is very similar to that of the sporocyst. It consists of a thin cuticula covering the external surface of the body, a layer of circular muscles followed by a layer of longitudinal muscles, and a thin epithelial layer. The cuticula gives a white appearance to the redia and is wrinkled in low transverse ridges by contraction of the body. The cuticula appears to be 4 to 6 μ thick in living specimens, but in well extended sectioned rediae it measures 1.5 to 2 μ in thickness. The fact that it is no thicker in the older forms than in young rediae indicates that there are no additions to it by underlying cells, and this condition supports the evidence concerning the transformation of the primitive epithelium into the cuticula.

This study of the developing and mature redia demonstrates that the development of this stage in the life history of *C. cotylophorum* is very rapid. The chronological sequence of organ formation and the time of germ ball formation is very similar to the same processes in the miracidium. The birth of the redia occurs 9 to 10 days after the snail host is

infested. All the structures are formed except the birth pore. The average size of rediae at the time of liberation from the sporocyst is 0.188 by 0.056 mm. Following liberation the redia reaches its maximum size in 5 days and may contain as many as 23 cercariae, all of which are retained in individual compartments. The germinal epithelium is located in the posterior extremity of the body and probably becomes exhausted after 25 cercariae are produced. The digestive system consists of a mouth, a pharynx, an esophagus, and a rhabdocoel intestine. The excretory system is similar to that of other amphistome rediae, consisting of an anterior, a median, and a posterior flame cell and their ducts on each side of the body. The nervous system consists of a mass of fibrous tissue and many associated ganglion cells located principally in the dorsal region of the body in the esophageal region. The body wall is composed of an outer cuticula, a circular and a longitudinal layer of muscles, and an inner epithelial lining. The average size of mature rediae is 0.59 by 0.163 mm and the birth pore is located on the ventral surface 0.168 mm from the anterior end of the body.

DAUGHTER REDIA

Daughter rediae have been reported for only three species of amphistomes. Looss (1896:184) mentions that daughter rediae occur in the life cycle of *Gastrodiscus aegyptiacus* and that occasionally both rediae and cercariae were found developing in the same mother redia. Looss (1896:189) also found that in the life cycle of *Paramphistomum cervi* two and sometimes three generations of rediae were produced. Beaver (1929:17) in his work on the life cycle of *Allasostoma parvum* found that daughter rediae were produced in mother rediae in which the pharynx and intestine were larger in proportion to the size of the body than in daughter rediae. He found, too, that the mother redia possessed only one pair of appendages and that the body was greatly distorted.

Only a single mother redia was found in the present material although many infested snails were examined over a period of nearly a year. This one specimen was found in a snail on August 4, 1934, one month after it was infested. It was not observed until too distorted by pressure to make accurate observations. However, it contained three well developed and three developing rediae. In the more developed individuals the digestive and excretory systems were fully formed and one or two germ balls were present. In this same snail many young cercariae were found in the liver as well as many other rediae which contained developing cercariae only.

CERCARIA

DEVELOPMENT

The cercaria acquires most of its structures while still within the redia, although some are represented by primordia only. In the experimental series under discussion mature cercariae were shed 32 days after the infestation of the snail. The first cercariae were freed from the rediae 15 days after infestation in a very immature condition. They were about one-third of the size of mature cercariae.

The first differentiation which occurs is the formation of the primitive epithelium, which does not develop until the germ ball or cercaria has reached a diameter of 30 μ . The appearance of the primitive epithelium is very similar to that surrounding developing rediae but it loses its cellular nature much more quickly than in the redia. No nuclei were distinguished in this layer in cercaria more than 60 μ in length.

The next structures to appear are the cystogenous gland cells. The first of these were found irregularly scattered through the body of a cercaria which measured 50 by 46 μ . These cells are easily recognized because of their size and characteristic appearance (Fig. 52). The cells are 8 to 10 μ and have nuclei 6 to 7 μ in diameter. A distinct deeply-staining chromatin body, 2 μ in diameter, is located in the nucleus. The remainder of the nucleus is finely granular while the cytoplasm is very transparent. The number of these cells increases rapidly as the cercaria develops.

Shortly after this stage in development the cercaria becomes distinctly ovoid, and at a length of 65 μ the primordia of the digestive and excretory systems appear. The excretory system consists of two lateral ducts which open separately at the posterior end of the body. Looss (1892:162) observed a similar condition in the developing cercaria of *Diplodiscus subclavatus* and was able to see flame cells at the internal ends of the ducts. In the present material no flame cells were seen prior to the birth of the cercaria. The digestive primordia consist of a group of cells located centrally near the anterior end of the body, in which lumina appear very early (Fig. 52). The most anterior lumen is that of the oral sucker and the most posterior that of the rhabdocoel intestine, which Looss (1892:163) considers as probably homologous to the intestine of the redia. The esophagus appears as a solid cord of cells connecting the oral sucker and the intestine. The number of cells entering into the formation of these primordia is much greater than in the redia. No cell boundaries could be distinguished but the number of cells present is indicated by the many small, closely packed nuclei.

Shortly after the formation of these structures a basal membrane in

which an occasional ovoid nucleus is located develops around them. The nuclei of this layer can be distinguished from those of the surrounding tissues by their position only. The ultimate development of this layer was not determined in the present material, but Looss (1892:165) states that these cells are derived from body parenchyma and give rise to the muscle layers surrounding these organs.

A mass of deeply-staining cells located immediately posterior to the blind end of the intestine at this stage in development represents the primordia of the male and female genital systems. The cells of this mass can be distinguished from surrounding cells by their position and staining reaction only, but by following them in their subsequent development their function is discovered.

In embryos of approximately $90\ \mu$ in length the tail is formed as a broad but short part of the body at the posterior end, which becomes set off rapidly from the body by ventral and lateral constrictions. The cells entering into the formation of the tail are similar in every respect to those of the body. As the constrictions deepen the excretory vessels in the tail become confluent, but retain their separate openings, which become lateral in position because of the narrowing and lengthening of the tail (Fig. 57). The union of the excretory ducts occurs first in the tail but continues into the posterior part of the body for a short distance. As a result of this fusion the excretory system consists of a tail duct with its two lateral openings and a lateral duct on each side of the body. At the junction of the tail duct and the two lateral ducts another pore is formed in the median dorsal line of the body. This is the pore of the future excretory bladder in both the mature cercaria and the mature worm. This pore is formed prior to the birth of the cercaria.

While these changes are taking place in the excretory system the digestive system is becoming complete. The rhabdocoel intestine bifurcates and a mass of cells is formed at the end of each fork which grows laterally and posteriorly on each side. These cells are the primordia of the caeca (Fig. 55). As the caeca elongate a small lumen is formed in them. They develop dorsal to the excretory ducts.

At this stage of development the cuticula-producing cells of the oral sucker still retain their nuclei and the mouth is not yet open. The lumen of the esophagus is present and the esophagus is lined by a thin cuticula continuous with that of the oral sucker. The cuticula lining both the esophagus and the oral sucker is thrown into longitudinal folds which are continued a short distance anterior to the oral sucker. It is possible that the mouth opening is formed in the cercaria as in the redia but no observations were made on the details of this process. However, the mouth opening is formed before the cercaria is born, being similar in

this respect to the redia, but the nuclei of the cuticular cells are retained until after birth. Looss (1892:164) assumes that the primary cuticula is sloughed by the cercaria as in the redia but he made no observations on this process. In the present material no sloughing was observed and it is believed that the primary cuticula produced by the primitive epithelium and the cells lining the oral sucker is retained throughout the life of the individual.

Simultaneously with the formation of the tail the nervous system and eyes appear. The nervous system at this stage of development consists of a small ganglion on each side of the esophagus. These ganglia are connected by a commissure passing dorsal to the esophagus immediately posterior to the primordium of the oral sucker. Several small nerves can be traced a short distance from each of the ganglia (Fig. 57). Many nuclei were observed arranged in a close series which completely encircle the ganglia and commissure clearly delimiting them from the surrounding parenchyma cells. Looss (1892:164) described a similar nervous system for the developing cercaria of *Diplodiscus subclavatus* and was able to trace the nerves much farther than could be done in the present material. At the time of the formation of the nervous system and the tail the cells producing the eyes become evident. The pigmented part of each eye is produced from a single cell located laterally above each ganglion. These cells could not be distinguished from the surrounding cells prior to the formation of the pigment. After the formation of the pigment the cells are conspicuous, having the appearance shown in Fig. 64. At this stage in development the cell measures 10 to 12 μ in diameter and the nucleus 6 μ . The nucleus contains a large central chromatin mass 3 μ in diameter. Looss (1892:165) observed three cells which contributed to the eye formation in the early developmental stages of the cercaria of *Diplodiscus subclavatus*. As he pointed out, one cell produced the pigmented part and the other two the lens of the eye. These latter two cells were not identified in the present material prior to the birth of the cercaria and then were recognized in sectioned material only (Fig. 65). These cells lie against the cuticula, between it and the pigment cell, and seem to give rise to a refractive substance which fills the space in the pigment cone. The arrangement of these cells was determined in cercariae which had a body length of 130 μ .

Thus in the cercaria the following structures can be identified before it leaves the redia (in order of appearance): primitive epithelium, cystogenous glands, excretory and digestive primordia, reproductive tissues, nervous system, pigment cells of the eyes, and the tail. The only structure which develops after birth and of which there is no indication before birth is the acetabulum.

Birth occurs shortly after the formation of the tail but the size at which the cercariae are born is not uniform. The approximate size of the body is 120 by 55 μ and of the tail 37 by 33 μ . The cercaria is capable of very weak movement which is sufficient to break the enclosing strands of tissue but the process of birth was never observed. The mouth is open and the caeca which extend the length of the body are provided with a small lumen, and so it is possible for the cercaria to begin feeding at once. Immediately after birth the eyes rapidly become more prominent and the other structures of the body also increase rapidly in size (Fig. 56); the primordia of the reproductive systems divide, forming two large masses which remain connected by a small cord of cells.

The acetabulum first appears as a solid mass of cells at the ventro-posterior surface of the body in cercariae approximately 140 by 65 μ . This mass projects prominently and measures about 45 μ in width and 30 μ in length. A lumen becomes conspicuous in the acetabulum when the cercaria has reached a size of 190 by 75 μ . At this stage the acetabulum appears as a wide flat mass of cells with a small concavity near its center. It is 50 μ wide and 20 μ thick.

Shortly after birth the eyes acquire a very heavy pigmentation and their characteristic oval shape which is retained until the cercaria is 150 μ in length. Pigment then begins to grow out from the eyes in lateral finger-like projections which completely encircle the body. A short time later projections are formed at the posterior surface also. At the same time the eyes become surrounded by a solid mass of pigment which entirely obscures their original outline (Fig. 61). As the growth of the pigment continues it is arranged in an irregular dendritic pattern over the dorsal and lateral surfaces of the body (Fig. 61). The branches break up into small irregularly arranged patches which remain attached to each other by very small strands of pigment. A short time before maturity is reached only a few large branches of pigment, which extend laterally and posteriorly from the eyes, are present (Fig. 59). The eyes assume their original shape but remain connected by a conspicuous band of pigment. It is possible that these later concentrations of pigment lie directly above the large nerves of the body as suggested by Sewell (1922:75). In the mature cercaria all of the pigment is uniformly distributed, there being no definite concentrations into lines or large patches as in the developing forms. Consequently the eyes, which retain their pigmentation, become very conspicuous.

The pigment is entirely superficial in position, being located immediately beneath the cuticula with the exception of small granules which are scattered throughout the body. The concentration of pigment on the

dorsal and lateral surfaces is much heavier than on the ventral surface, but no area was found entirely devoid of pigment.

Simultaneously with the formation of the acetabulum the two lateral excretory ducts become united by a cross connection located across the middle of the body near the dorsal surface. The lateral excretory ducts pass outward and forward from their union with the caudal duct, then turn forward and inward and at their most inward position the cross connection is formed. From this point they turn outward and forward and pass ventral and mesial to the eyes to reach the sides of the oral sucker. Here they turn sharply and pass back toward the posterior end of the body. In cercariae between 150 and 175 μ in length a small median anterior diverticulum is formed on the cross connection and a lateral diverticulum grows out from each lateral duct immediately posterior to the eyes. The excretory system is shown in Fig. 50.

As has been stated previously an excretory pore is formed dorsal to the union of caudal and lateral excretory ducts prior to the birth of the cercaria. The excretory duct of the bladder is lined for a considerable distance by cuticula, which makes it evident that the pore is formed by an invagination of the body wall which comes in contact with the excretory ducts at their union. As the cercaria increases in length the posterior ends of the lateral ducts become situated more posteriorly. This change in position is slight but necessitates an anterior extension from their point of union to the excretory pore. At first, in cercariae of not over 0.2 mm in length, this anterior extension is no wider than the ducts, but as growth continues it assumes the appearance of the bladder or excretory vesicle in the mature cercaria. The lateral ducts empty at all stages of development into the posterior lateral margins of the bladder. The caudal excretory duct opens into the excretory duct from the bladder (Fig. 45). Consequently, the bladder is formed by first an extension and later an expansion of the ends of the lateral excretory ducts at their point of union.

The cystogenous glands which increase in number as the cercaria develops begin to produce cystogenous rods or granules when the cercaria is approximately 140 μ in length. Previous to this stage in development the cercaria stains very deeply in both toto mounts and sectioned material, but with the formation of the cystogenous granules this staining reaction largely disappears. This is due to the fact that the granules are very difficult to stain. The increase in number of these granules is so rapid and the area they occupy is so great that in cercaria of 200 μ in length there is comparatively very little tissue in the body. The body parenchyma is almost completely obliterated, only an occasional nucleus surrounded by a small amount of cytoplasm being found. The cystogenous cells are

round and vary from 13 to 21 μ in diameter when filled with the granules. The rod-shaped granules are arranged in parallel series in the cells and measure 12 by 4 μ .

All of the structures of mature cercariae are present but not fully developed in individuals which measure approximately 0.175 by 0.09 mm. The structures continue to increase in size but marked changes occur only in the pigmentation, the size of the excretory bladder, and the size of the tail. The changes occurring in the first two have been discussed previously.

In its early stages of development the tail consists of a dense mass of cells set off from the body. It is much broader than long when first formed and at the time of the birth of the cercaria the dimensions are practically equal. It is also capable of very slight movements which consist of slow contractions and extensions. It becomes longer than wide immediately after birth, and the length increases rapidly. Both the body and tail of the cercaria are subject to considerable variation in size in older individuals, but in well extended fixed specimens the tail and body become approximately equal in length when both are about 0.2 mm long. Three or four days before maturity is reached the tail is sufficiently strong to enable the cercaria to swim for brief intervals. Such cercaria will swim for a short time and then apparently attempt to encyst but cannot. In cercaria of this age the tail is approximately twice the length of the body.

The number of cells comprising the tail appears to remain unchanged. They are very small and numerous in the young stages, as indicated by the nuclei, but become large in mature individuals (Fig. 54), giving the impression that the cells increase in size but not in number as the tail grows.

MATURE CERCARIA

Age at Birth.—Mature cercariae escaped from the snails in this experiment 32 days after infestation. The development of the cercaria when compared to that of the redia is very slow. It will be recalled that the first rediae were freed from the sporocyst in 9 days and at the time contained developing cercariae 5 days old. The cercariae continued to develop in the redia for another 5 days before the first of the cercariae were born at an age of 10 days. Following their birth these oldest cercariae developed for another 22-day period in the liver of the snail before they made their escape.

Size.—Because of their size and heavy pigmentation the free-swimming cercariae are readily distinguishable while swimming if they are placed over a white background. The cercaria is extremely variable

in form, and since it is constantly active exact measurements are hard to secure. When fully extended the body may be three times its width and very flat. When fully contracted the width is slightly greater than the length and the body may be one-half as thick as it is wide. When fixed in warmed Bouin's or corrosive sublimate fixatives the body contracts still more. In the contracted state the anterior end of the body is pulled ventrally.

TABLE 12.—MEASUREMENTS (IN MICRONS) OF TEN CERCARIAE EXTENDED, CONTRACTED, AND FIXED

No.	Body measurements			Tail measurements		
	Extended	Contracted	Fixed	Extended	Contracted	Fixed
1.....	302 x 151	151 x 235	168 x 184	670 x 47	352 x 100	336 x 84
2.....	302 x 252	201 x 201	252 x 184	655 x 60	302 x 84	369 x 84
3.....	336 x 168	168 x 252	176 x 189	672 x 50	420 x 84	378 x 71
4.....	386 x 117	189 x 231	218 x 184	588 x 50	302 x 84	554 x 67
5.....	403 x 108	186 x 240	218 x 184	630 x 50	302 x 84	554 x 67
6.....	420 x 134	235 x 268	235 x 210	588 x 60	319 x 84	420 x 71
7.....	431 x 156	245 x 303	216 x 216	621 x 45	312 x 89	353 x 55
8.....	451 x 184	196 x 235	176 x 196	686 x 58	294 x 98	333 x 59
9.....	460 x 156	196 x 274	187 x 187	706 x 54	392 x 89	460 x 54
10.....	490 x 134	235 x 314	319 x 268	672 x 70	470 x 100	420 x 84
<i>Average</i>	398 x 153	200 x 252	236 x 200	668 x 55	346 x 89	417 x 69

The tail size varies considerably also. When fully extended it may be approximately twice as long as when contracted, but when fixed it is usually slightly longer than when contracted.

The measurements made on 10 cercariae when extended, contracted, and fixed are given in Table 12. When the average length of the body and tail of the 10 are combined the total length of the cercaria is 1.066 mm when fully extended and only 0.546 mm when fully contracted. The same measurement on fixed specimens was 0.653 mm.

Body Wall.—In contracted specimens the surface of the body is covered by a thin cuticula in which is seen a series of clear longitudinal lines running parallel to each other. Sewell (1922:75) saw similar lines in *Cercariae Indicae* and thought that they might denote longitudinal muscle fibers. However, since these lines are superficial in position, large and distinct, and can be seen only in living contracted specimens (not being seen at all in toto mounts or sectioned material) it is believed that they are only folds in the cuticula.

Pigment.—The pigment of the body is distributed over the entire body in a solid layer which is about 3 times as thick on the dorsal and

lateral surface as it is ventrally. A few pigment granules are scattered irregularly throughout the body (Fig. 58).

Eyes.—The eyes are located far forward, lateral to and immediately posterior to the oral sucker (Fig. 51). They are conical in shape with the base located immediately beneath the cuticula and with the apex directed ventro-posteriorly in the body. The base is surmounted by a clear refractile lens in which are two nuclei representing the cells from which the lens is formed. The nuclei measure approximately 7 by 5 μ and each contains a small distinct chromatin body. The cone is 30 μ long and 18 μ in diameter at its base. When the cercaria is swimming the anterior end of the body is drawn ventrally, leaving the eyes located at the most anterior and dorsal point of the body.

Acetabulum.—The acetabulum in the relaxed condition in living cercaria measures 65 μ in diameter. In contracted, fixed, and sectioned material the acetabulum is very much smaller. It measures 43 μ in diameter under such conditions.

Digestive System.—An accurate study of the digestive structures cannot be made in either living cercaria or toto mounts because of the heavy pigmentation and the cystogenous glands. The extent of the esophagus cannot be determined and the caeca are not visible in living specimens. In toto mounts these structures can be seen indistinctly. The oral sucker is terminal and usually ovoid in shape, its length slightly exceeding its width. Its average size in living cercariae is 47 μ in length and 46 μ in width; in fixed sectioned material the average size is 32 by 32 μ . When the oral sucker is retracted a distinct oral cavity is formed which is lined by smooth cuticula.

The esophagus originates near the ventral surface of the oral sucker and passes dorsally and posteriorly (Fig. 58). It bifurcates posterior to the eyes and in the dorsal part of the body to form the intestinal caeca. It is lined by a cuticula continuous with that of the oral sucker. The walls of the esophagus are thin at its origin but increase in thickness posteriorly. There is no definite sphincter muscle at its termination but the increase in thickness gives it a bulbous appearance. Deeply-staining glandular cells, the esophageal glands, completely surround the esophagus throughout its length (Fig. 47).

The caeca are small, measuring 15 μ in diameter, and terminate above the anterior margin of the acetabulum.

Nervous System.—No parts of the nervous system can be seen in living specimens or toto mounts and only the larger parts can be traced through sections. The central nervous system consists of two ganglia connected by a transverse commissure. The ganglia are located directly

beneath the eyes and the commissure passes across the body dorsal to the esophagus, immediately posterior to the dorsal surface of the oral sucker. The ganglia are approximately $15\ \mu$ in diameter and the commissure is $9\ \mu$ in diameter in the median line of the body. Each ganglion gives rise to several nerves. Three were found to pass anteriorly and terminate on the body surface lateral to the oral sucker, two being ventral and one dorsal. Another nerve passes dorsally and terminates in contact with the inner end of the eye (Fig. 66). A posterior nerve passes from the ganglion to the ventral surface of the caecum on the same side where it disappears from view. The ganglia, the commissure, and the nerves are entirely enclosed by numerous small nuclei which probably represent nerve cells.

Reproductive System.—The primordia of the male and female reproductive systems are distinguishable in the mature cercaria. These primordia consist of two large groups of cells connected by a single cord of cells (Fig. 58). The most posterior mass which represents the ovary, Mehlis' gland, and testes is located immediately dorsal to the anterior margin of the acetabulum. It consists of numerous deeply-staining cells and nuclei. This mass may vary from 22 to $29\ \mu$ by 16 to $20\ \mu$ in contracted specimens. From it a cord of cells passes anteriorly in the center of the body which connects with the second large group of cells located immediately posterior and slightly ventral to the intestinal bifurcation. From its ventral side a cord of cells passes ventrally, terminating against the surface of the body. Its point of termination marks the position of the genital pore but no opening could be found in the cercaria. The cells of these primordia are entirely similar.

Excretory System.—The excretory system of the mature cercaria is essentially the same as in the immature cercaria. The presence of the heavy pigmentation and the cystogenous glands makes it impossible to determine the flame cell pattern or the position of the smaller excretory tubules. Sixteen flame cells were found in developing cercariae irregularly distributed but principally around the acetabulum and oral sucker prior to the spreading of the pigment to these regions. Consequently, only the larger units are described here.

The excretory bladder is located dorsal to the acetabulum and the posterior genital complex (Fig. 58). A short cuticula-lined duct passes from its anterior end dorsally to the excretory pore located in the dorsal median line above the anterior margin of the acetabulum. In extended cercariae there may be a slight change in these relationships. In such specimens the duct passes dorso-anteriorly from the bladder and the excretory pore may be as much as 10 or $15\ \mu$ anterior to the acetabulum. Two large excretory ducts join the posterior lateral margin of the bladder. From

this point they pass outward and forward for a short distance and then turn inward. Slightly posterior to the middle of the body the cross connection previously described passes across the median line and sends off a small median diverticulum. The main ducts are ventral to the caeca but the cross connection passes dorsally and the diverticulum is located near the dorsal surface of the body a short distance posterior to the intestinal bifurcation. No tributaries were seen emptying into this diverticulum. Anterior to the cross connection the main ducts turn outward and then turn inward again posterior to the eyes. They pass forward ventral to the eyes and ganglia to reach the sides of the oral sucker. Here they turn sharply and pass back to the posterior end of the body, terminating near the acetabulum. Diverticula are formed on the main ducts also immediately posterior to the eyes. As in the median diverticulum no ducts were seen opening into them.

The main ducts from the bladder to the oral sucker, the cross connection, and the diverticula were filled with excretory granules varying from 2 to 10 μ in diameter. The smaller granules were located in the extremities of the ducts while the larger ones were located in the main ducts near the center of the body.

The wide caudal excretory tube joins the excretory duct just before it reaches the pore. It passes backward through the center of the tail and enlarges to form a small bladder at its posterior end a short distance from the end of the tail. From this enlargement a short duct passes posteriorly and laterally on each side to reach the surface. These ducts are the ends of the two original excretory ducts of the very young cercaria but the presence or absence of an opening for these was not determined for older ones.

Free-Living Cercariae.—It was observed that these cercariae escaped from the infested snails during a definite period each day and at no other time unless artificially stimulated. The time and numbers of cercariae escaping from individual snails are shown in Table 13. The snails used in this experiment were infested on May 5, 1934, and began shedding cercariae on June 5, 1934. There were 35 snails in this group, all of which were shedding cercariae at the same time, but only 15 were used to illustrate the periodicity of shedding.

Five snails were placed in individual finger bowls which contained a small amount of water and a piece of lettuce. The size and pigmentation of the cercariae makes them easy to see, especially when placed over a white background. It had been observed previously that the snails would shed cercariae at any time if they were kept out of water for as long as 24 hours and then put back into water. The first snails used in this experiment were taken from an aquarium (in which water was present at

all times) at 8:00 A.M. on June 14 and placed in the finger bowls. The cercariae began escaping a short time after 11:30 A.M. and continued to escape until 5:30 P.M. The snails were under observation continuously and the cercariae were removed as they emerged. Group 1 of Table 13 shows the results of this experiment. The snails were kept under observation in the bowls until 2 A.M. June 15 but no more cercariae escaped until about noon of the following day. A similar experiment was made with 5 other snails on June 15 and June 19. The results indicate definitely that cercariae escape in largest numbers during the brightest hours of the

TABLE 13.—SHOWING PERIODIC SHEDDING OF CERCARIAE BY THREE DIFFERENT GROUPS OF FIVE SNAILS EACH

Time	Group I					Group II					Group III				
	June 14, 1934					June 15, 1934					June 19, 1934				
10:30-11:30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11:30-12:30	55	5	104	0	5	310	7	32	32	14	126	125	18	0	138
12:30-1:30..	4	88	101	0	0	9	170	4	16	23	39	45	0	0	4
1:30-2:30...	0	0	0	90	0	21	46	2	8	1	14	0	0	0	0
2:30-3:30...	0	27	0	1	0	1	2	0	0	0	16	0	0	0	0
3:30-4:30...	0	1	32	4	0	0	0	0	0	0	1	0	0	1	0
4:30-5:30...	0	0	12	0	0	0	0	0	0	0	5	0	0	0	0
5:30-6:30...	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total....	59	119	249	95	5	341	225	38	56	38	201	170	18	1	142

day. June 15 was a rainy day and much darker than either June 14 and 19, and cercariae escaped through a period of 3 hours only, while on the other days which were very bright this period increased to 6 hours in some instances. This response of the cercariae to light is demonstrated by free-swimming cercariae also. Immediately after escaping they collect in the dish where the light is most intense, and in a short time begin to encyst at the surface of the water. If vegetation is placed in the dish the majority of the cercariae encyst on the part at which the light is most intense. However, they select the vegetation for encystment in preference to the glass even though the light is less intense near it. This response to light is further demonstrated by the fact that cercariae make their escape at any time of the day if the snails are placed directly beneath a strong light. This response is most easily demonstrated in the following manner. If a number of cercariae are drawn into a pipette which is then held perpendicular to the edge of a lamp shade covering a lighted electric lamp with the pipette exposed to the light, the cercariae move back and forth in it. If the pipette is then drawn past the shade so that the upper end is darkened the cercariae move into the lighted area and never leave

it for more than a few seconds. By continuing to raise the pipette the cercariae can be concentrated at the end of the pipette. This method of concentration is rapid and useful in securing large numbers of cercariae in a minimum amount of water.

The cercariae are active swimmers but do not swim for long periods. After emerging from the snail they swim in a small circle for a short time and then proceed to the most illuminated part of the dish. They swim for a few seconds and then drop to the bottom of the dish or come to rest on small bits of debris or vegetation for a short time. At the sides of the dish they swim at the surface briefly then settle slowly to the bottom but are soon back at the surface again. This activity may continue for as long as an hour if there is no vegetation on which to encyst but none were found to remain unencysted for longer periods unless constantly disturbed. If vegetation is present some cercariae begin to encyst at once but others require as long as 30 minutes. They encyst readily on many kinds of vegetation but lettuce was used in most instances. If cercariae are placed in the hollow of a lettuce leaf they collect in the small folds of the leaf but always on the side turned toward the light.

The number of cercariae escaping from the snails (Table 13) was observed to vary considerably. If a snail produced a large number of cercariae in one day, it was 8 days before any more escaped in some instances. However, the time interval may be as short as one day, but the number emerging on the second day is always small. That this variation is not due to a loss of infestation was demonstrated by dissecting some of the snails shown in Table 13 after an interval of 4 or 5 days during which no cercariae were shed. In every snail examined many developing rediae, mature rediae, and developing cercariae were found in large numbers.

Cort (1922:183) observed that cercariae escaped at definite periods each day and that these periods remained the same for specific cercariae but differed for different species. He also observed that there was considerable variation from day to day in the numbers produced. Krull and Price (1932:20) observed that the cercariae of *Diplodiscus temperatus* escaped at any time of the day but that largest numbers escaped between 11:00 A.M. and 5:30 P.M. They also observed that there is a very definite heliotropic response in these cercariae. Krull (1934:175) found that the cercariae of *Cotylophoron cotylophorum* escaped from the snail host between 8:30 A.M. and 1:00 P.M., the peak being about 8:30 A.M. This observation shows that the cercariae escaped periodically, but the time at which the largest numbers are shed is quite different from the findings in the present observations on the same species. Krull also found that these cercariae occasionally escaped at other hours of the day.

Krull and Price (1932) and Krull (1934) noted that the number of cercariae escaping from the snails increased the length of the period of escape. That this was not the case in the present experiments can be seen from the results shown in Table 13. Several instances are shown in which a larger number of cercariae escaped in less time than that required for the escape of smaller numbers from other snails.

The length of time snails remain infested was not determined. However, the time would depend on the rate of development of the various stages. In the present work snails infested during the coldest months of the year shed cercariae only after 91 days, and in the warmest months cercariae were shed 30 days after infestation of the snails. That a definite number of rediae is produced by each sporocyst and a definite number of cercariae is produced by each redia has been pointed out. Consequently the snails do become free of infestation but only after a prolonged period. Under optimum developmental conditions the sporocyst remains productive for fully 30 days, the redia for another 30 days, and the cercaria requires 22 days to develop after leaving the redia. Thus under theoretical optimum conditions snails remain infested for at least 78 days and probably remain infested under natural conditions for periods ranging from 4 to 6 months, which is doubtless equal to or exceeds the life of the snail. Krull (1934:175) was able to keep infested snails alive for 6 weeks after cercariae began to escape, 36 days after infestation. The number of days these snails remained alive after infestation, 76 days, is very close to the theoretical time limit they will remain infested under optimum conditions. Krull's work was done in the summer months of May, June, July, and August which may be considered as the optimum developmental period for the parasite.

DISCUSSION OF PREVIOUSLY DESCRIBED AMPHISTOME CERCARIAE

Sixteen species of amphistome cercariae have been described, two of which have been considered doubtful. Cary (1909:604-607) described an amphistome cercaria which he considered to be that of *Diplodiscus temperatus*. However, Cort (1915:23-30) has pointed out that Cary was mistaken in considering the cercaria he studied as being that of *D. temperatus*. Ward (1916:17-19) described *Cercaria gorgonocephala* and expresses his own uncertainty as to its exact systematic position, and as it is not closely related to the cercaria of *C. cotylophorum* it needs no further consideration here.

Looss (1892:162-166) described in detail the cercaria of *Diplodiscus subclavatus*. Later he (1896:185-191) described the cercariae of *Paramphistomum cervi* and *Gastrodiscus aegyptiacus* (1896:177-185). His

conclusions in the latter case, however, rest on the structural comparison of the cercaria and the adult.

Cort (1915:24) described *Cercaria diastrophia* and *C. inhabilis* and called attention to the fact that the 5 species of amphistome cercariae then described belong to two subfamilies of the Paramphistomidae. He assigned the cercaria of *Paramphistomum cervi* to the subfamily Paramphistominae; and *Cercaria diastrophia* and *C. inhabilis*, the cercaria of *Diplodiscus subclavatus*, and that of *Gastrodiscus aegyptiacus*, he assigned to the subfamily Diplodiscinae. Sewell (1922:66) accepted Cort's classification and added 3 new species, *Cercariae Indicae* xxvi, xxix, and xxxii to the former group; and *C. frondosa* Cawston (1918), *C. corti* O'Roke (1917:165-180) and a new species *C. Indicae* xxi, to the latter. Sewell omitted *C. convoluta* Faust (1919:172), but Faust states that it probably belongs to the Diplodiscinae.

Sewell (1922:67, 80) prepared a key for the separation of two distinct groups for which he proposes the names "Pigmentata" and "Diplocotylea." He assigned the cercaria of *Paramphistomum cervi*, *Cercariae Indicae* xxvi, xxix, and xxxii to the former group, and the other cercariae mentioned above he assigned to the latter group. To this latter group, Diplocotylea, McCoy (1929:200) added *Cercaria missouriensis*. Beaver (1929:20) described the cercaria of *Allasostoma parvum* and assigned it to the Diplocotylea also, and he points out that *A. parvum* belongs to the subfamily Schizamphistominae Looss 1912. He further points out the mistake made by Cort (1915:24) in assigning amphistome cercariae to subfamilies based on larval characteristics only, since the diagnostic characteristics of amphistomes are restricted to characters which are not present in the larval forms. His conclusion that only Séwell's classification can be rightly used is considered to be entirely correct.

C. cotylophorum belongs to the Paramphistominae, so that the present cercaria may be assigned to that subfamily. The cercaria possesses all of the characteristics of the "Pigmentata" and can be assigned to that group also.

This cercaria can be readily distinguished from that of *P. cervi* by its much smaller size, shorter tail, smaller suckers, and particularly by the presence of the evaginations from the excretory vessels. These evaginations are lacking in the cercaria of *P. cervi*.

Cercariae Indicae xxvi differs from the present cercaria in that *C. Indicae* is very much larger; the suckers are nearly twice as large; the esophagus is narrow without any trace of a pharynx or sphincter muscle; the genital system is well developed and shows a differentiation into the

respective organs, and the vitelline glands are well developed. In view of these differences there can be no doubt that the two cercaria are entirely different.

Cercariae Indicae xxix presents fewer differences than the two above but can be distinguished from the present cercaria by a few characteristics of diagnostic value. This cercaria is slightly larger than the cercaria of *C. cotylophorum* yet possesses suckers almost twice as large; there is no thickening of the circular muscle layer around the esophagus, and the "anlage" of the vitelline glands are present. Consequently, these two cercariae must be considered as different species.

The only other cercaria assigned to the "Pigmentata" group is *C. Indicae* xxxii. This form is readily distinguished from the present cercaria also. *C. Indicae* xxxii is slightly larger and possesses an oral sucker 3 times as large, and an acetabulum more than twice as large as these structures are in the cercaria of *C. cotylophorum*. It differs also in possessing a very long esophagus with a well marked sphincter muscle at its posterior end, and in that the excretory pore opens posteriorly.

It is quite evident from a comparison of these forms that the cercaria of *C. cotylophorum* has not been described previously. Le Roux (1930: 247) mentions finding an amphistome cercaria in a snail, *Bulinus schakoi*, which he considers as practically identical with *C. frondosa* Cawston as described by Faust (1919:172), and which he thinks is the cercaria of *C. cotylophorum*. He did not describe it, however, and it is impossible to determine what cercaria he found. Faust describes *C. frondosa* as having pharyngeal pockets and does not describe the cross connection between the excretory ducts which is characteristic of the group to which the cercaria of *C. cotylophorum* belongs. The possession of pharyngeal pockets and the absence of the connecting duct clearly places *C. frondosa* in the "Diplocotylea" group. Consequently, if the cercaria found by Le Roux were *C. frondosa* it was not the cercaria of *C. cotylophorum*.

METACERCARIA

Cercariae remain free-swimming from 10 minutes to an hour after escaping from the snail, but the usual time for them to remain unencysted when vegetation on which to encyst is present is from 20 to 30 minutes. When encystment begins the cercaria actively elongates and contracts the body while the tail is beating violently. This movement alternates with one in which the body is thrown from side to side by the movements of the tail as in swimming, but no progress is made. At the same time the cystogenous material is breaking down and flowing out all over the body surface. The cuticula seems to loosen and the body contracts until a con-

siderable space is left between it and the cuticula. The cystogenous material collects on this membrane, producing at first a roughened surface both externally and internally. The cercaria is constantly in motion during this process, twisting the body from side to side. These movements smooth the internal wall of the cyst and distribute the cystogenous material evenly. The tail continues to beat rapidly, and as the cyst is formed it becomes loosened from the body but may remain attached to the cyst wall for some time. When freed from the cyst it swims away and continues to swim for as long as 8 hours. The process of encystment is usually complete after 20 minutes but the metacercaria may continue to contract from side to side for a much longer period.

The completed cyst is round in surface view, and dome-shaped in lateral view, since it is slightly flared at the base (Fig. 53). The base is hollow and acts as a support for the remainder of the cyst. The cyst proper averages $154\ \mu$ in diameter, and the base $184\ \mu$; the total height averages $130\ \mu$. The cyst wall is transparent but appears whitish to the unaided eye. The metacercaria retains its pigmentation, and the only structures even slightly visible are the oral sucker, the acetabulum, the eyes, the excretory pore, and the longitudinal striations in the cuticula described as being present in the contracted cercaria.

Under optimum conditions the metacercaria probably lives for several months as was pointed out by Krull (1934:176). To determine the longevity of the metacercaria 50 cercariae were allowed to encyst on the bottom of small dishes which were set aside and covered. Encystment occurred on June 5, 1934. On August 5, 1934, 56% were alive, and on September 5, 1934, 33% were alive. This experiment was carried no further but it is evident that under optimum natural conditions the metacercariae may live as long as 5 or 6 months. Krull kept them alive for 5 months under experimental conditions.

ADULT

EXPERIMENTAL INFESTATION

Development of Amphistome Parasites in the Final Host.—The information on this subject is very meager, consisting of chance observations and of only one concluded experimental problem. Looss (1892:166) observed that *Diplodiscus subclavatus* develops slowly in frogs during the winter months but he did not determine the time required for the worms to become mature. Beaver (1929:13) observed that a cercaria from *Planorbis trivolvis* was found to encyst on crayfish and frog larvae, and when fed to bullfrogs (*Rana catesbiana*) and snapper turtles (*Chelydra serpentina*) developed into a known species, *Allassostoma parvum*

Stunkard 1916. However, Beaver did not determine the time required for this species to become mature. Krull and Price (1932:34) determined experimentally that in lightly infested frogs *Diplodiscus temperatus* reaches maturity in 27 days but that in most hosts 2 or 3 months is the usual time required. Le Roux (1930:248) states that young forms of *C. cotylophorum* probably live in the duodenum of the final host 6 to 8 weeks before migrating into the rumen, where they become mature in another 6 to 8 weeks. Le Roux's statement was not supported by experimental evidence. Krull (1934:177) determined experimentally that *C. cotylophorum* matures in the latter part of the fourth month after

TABLE 14.—SHOWING RESULTS OF EXPERIMENTS IN WHICH CALVES WERE FED METACERCARIAE OF *Cotylophoron cotylophorum*

Host	Date of infestation	Date of examination	Number of metacercariae fed	Number of worms in duodenum	Number of worms in rumen
Calf I.....	Nov. 19, 1933	Jan. 4, 1934	150	0	6
Calf III.....	June 4, 1934 June 11, 1934 June 18, 1934	Jan. 25, 1934	300 300 300	199	4
Calf IV.....	June 4, 1934 June 11, 1934 June 18, 1934 June 25, 1934	July 25, 1934	300 300 300 300	19	34

infestation. His determination was based on the findings of eggs in the faeces of the host. Krull did not attempt to determine the age or size at which the parasite migrates from the duodenum to the rumen nor the size at which the parasite becomes mature after reaching the rumen.

Experimental Infestation of the Final Host of C. cotylophorum.—On June 28, 1933, two 7 or 8 months old calves were received at the animal pathology department of Louisiana State University. These animals were kept in a dry, well ventilated room when being used for experimental purposes but were allowed to graze in a small grassy plot at other times. No snails were present in this area so that there was no opportunity for the calves to become infested by *C. cotylophorum*.

These calves were obtained for experimental work in connection with this problem early in November, 1933. They were placed in the room previously mentioned on November 10, where they remained until the end of the experiment on January 4, 1934. The faeces of both calves were examined several times for the eggs of *C. cotylophorum* but were found to be negative. On November 19, 1933, they were separated by a

partition placed in the room, and Calf I was fed 150 metacercariae. Calf II was kept as a control (Table 14). Subsequently both animals were given the same type of feed and were given water from the same source.

TABLE 15.—SIZE (IN MILLIMETERS) OF *Cotylophoron cotylophorum* IN RUMEN OF EXPERIMENTAL CALVES

Note: In Calf I, infested 46 days, 6 worms were found in the rumen; in Calf III, infested 21 days, 4 worms; in Calf IV, infested 51 days, 34 worms (sizes of 20 are given here).

No.	Calf I	Calf III	Calf IV
1.....	3.25 x 1.71	2.06 x 0.63	1.95 x 0.78
2.....	3.37 x 2.13	2.10 x 0.63	1.95 x 0.91
3.....	3.55 x 2.15	2.29 x 0.73	2.08 x 0.72
4.....	3.60 x 2.20	2.95 x 0.84	2.08 x 0.85
5.....	3.67 x 2.08	2.13 x 1.04
6.....	3.85 x 2.45	2.28 x 0.85
7.....	2.31 x 0.91
8.....	2.34 x 0.80
9.....	2.34 x 0.96
10.....	2.34 x 1.04
11.....	2.47 x 0.85
12.....	2.54 x 0.78
13.....	2.60 x 0.78
14.....	2.60 x 0.85
15.....	2.60 x 0.91
16.....	2.60 x 0.98
17.....	2.62 x 0.85
18.....	2.70 x 0.83
19.....	2.73 x 0.88
20.....	2.86 x 0.99
Average.....	3.55 x 2.32	2.32 x 0.71	2.41 x 0.84

The faeces of both calves were examined at 2-day intervals after the twenty-first day of infestation. However, no eggs were found. Both animals were killed on January 4, 1934, after 46 days of infestation, in an attempt to establish the time of migration of the parasites. No parasites were found in Calf II, but 6 small mature specimens of *C. cotylophorum* were obtained from the rumen of Calf I. None was present in the duodenum. The parasites recovered varied in length from 3.25 to 3.85 mm and in width from 1.71 to 2.45 mm (Table 15). Only a few eggs were present in the uterus and only a few were deposited by the worms, although they were kept alive in physiological salt solution for 4 hours.

The presence of mature specimens in Calf I indicates that maturity is reached in less than 46 days, or that the worms were present as a result of natural infestation before the calf was confined and were depositing so few eggs that they were missed in the faecal examinations. The latter supposition is doubtless correct in view of the fact that Krull

(1934:177) determined in a series of experiments that this parasite becomes mature in approximately three and one-half months. Unfortunately, Krull did not determine the size at which this species becomes mature nor does he give the size of the worms recovered from the rumen of one of his experimental animals. However, Krull kindly loaned me one toto mount and one specimen serially sectioned, and a comparison of the present material to his is possible. Krull's specimens were approximately 6 months and 20 days old and the present material was 6 months and 7 days old, if we assume that Calf I became infested a short time before being confined. A comparison of size of the oral sucker, the esophageal thickening, the acetabulum, the testes, ovary, Mehlis' gland, and the

TABLE 16.—SHOWING NUMBER, SIZE (IN MILLIMETERS), AND DISTRIBUTION OF *Cotylophoron cotylophorum* IN THE DUODENUM OF EXPERIMENTAL CALF III

No.	1st foot (54 present)	2nd foot (40 present)	3rd foot (34 present)	4th foot (14 present)	5th foot (6 present)	6th foot (12 present)
1.....	1.22 x 0.72	0.99 x 0.36	1.35 x 1.05	2.08 x 0.78	1.61 x 0.39	1.56 x 0.80
2.....	1.50 x 0.95	1.43 x 0.85	1.43 x 0.75	2.26 x 0.99	1.90 x 1.06	1.97 x 1.06
3.....	1.69 x 0.93	1.45 x 1.05	1.43 x 0.85	2.39 x 0.80	2.50 x 0.93	2.26 x 0.85
4.....	1.60 x 0.80	1.69 x 0.96	1.71 x 1.04	2.60 x 0.78	2.54 x 1.04	2.54 x 0.91
5.....	1.90 x 0.95	1.85 x 1.10	2.13 x 1.04	2.67 x 1.01	2.75 x 1.01	2.60 x 1.04
6.....	2.00 x 0.85	2.13 x 1.04	2.30 x 1.15	2.73 x 0.93	3.09 x 1.04	2.75 x 0.96
7.....	2.00 x 1.05	2.25 x 0.75	2.50 x 1.25	2.75 x 1.01	2.75 x 1.01
8.....	2.05 x 1.10	2.25 x 0.80	2.70 x 1.05	2.76 x 1.04	2.75 x 1.14
9.....	2.15 x 1.05	2.55 x 0.95	2.75 x 1.15	2.86 x 1.06	2.99 x 1.04
10.....	3.05 x 0.95	2.60 x 1.10	2.80 x 1.05	2.99 x 1.01	3.09 x 1.04
Average..	1.94 x 0.94	1.95 x 0.90	2.11 x 0.96	2.61 x 0.94	2.23 x 0.90	2.63 x 0.99

genital sucker of Krull's sectioned material and similar structures in the smallest of the worms from Calf I demonstrates that the specimens are approximately the same age. The finding of no immature specimens in either the rumen or the duodenum indicates that none of the metacercariae fed to the calf developed.

This experiment was repeated in another attempt to determine the time of migration of parasites from the duodenum to the rumen. Two 4 months old calves which had not had access to infestation were obtained and kept under conditions similar to those of the first experiment. Calf III was fed 300 metacercariae on June 4, 1934, 300 on June 11, and 300 on June 18, making a total of 900 fed at 7-day intervals. Calf IV was fed a total of 1200 metacercariae at 7-day intervals from June 4 to June 25.

Calf III was examined on June 25, 1934, 21 days after the first infestation, and 203 immature specimens of *C. cotylophorum* were recovered. Of these, 199 were distributed in the anterior 6 feet of the

duodenum and 4 were found in the rumen. The other parts of the stomach were examined but no parasites were found. The smallest specimen from the duodenum measured 0.99 by 0.36 mm and the largest was 3.09 by 1.04 mm. There was no distinct grouping according to size so that it was impossible to determine the exact age of any one specimen. However, it may be safely assumed that the smallest worms were only 7 days old, whereas the largest were 21 days old. The large and small individuals were distributed irregularly in the duodenum so that it was again impossible to group them according to their distribution (Table 16). However, the largest number of small specimens was found in the upper 3 feet of the duodenum, indicating that the metacercariae probably excyst in this region. Most of the worms distributed posteriorly were much

TABLE 17.—SHOWING NUMBER, SIZE (IN MILLIMETERS), AND DISTRIBUTION OF *Cotylophoron cotylophorum* IN THE DUODENUM OF EXPERIMENTAL CALF IV

No.	1st foot	2nd foot	3rd foot	4th foot	5th foot
1.....	2.13 x 1.04	1.71 x 0.78	2.21 x 0.88	1.69 x 0.83	2.41 x 0.80
2.....	2.31 x 0.96	2.08 x 0.78	2.34 x 0.88	2.36 x 0.91
3.....	2.34 x 0.91	2.62 x 0.72	2.34 x 0.91
4.....	2.36 x 1.04	2.39 x 0.83
5.....	2.41 x 0.96
6.....	2.52 x 0.96
7.....	2.54 x 0.85
8.....	2.60 x 1.09
9.....	2.73 x 0.85
Average.....	2.28 x 0.99	1.60 x 0.76	2.44 x 0.91	0.02 x 0.87	2.41 x 0.80

larger than the smallest worms, indicating that the worms either migrate after becoming excysted anteriorly or that some metacercariae are carried farther before being liberated. The average size of the 199 specimens was 2.28 by 0.94 mm.

The 4 specimens found in the rumen of this host indicate that migration begins at the end of the first 3 weeks after infestation. These specimens varied from 2.06 to 2.95 mm in length and from 0.63 to 0.84 mm in width (Table 15). The average size of the 4 was 2.32 by 0.71 mm.

Calf IV was examined on July 25, 1934, 51 days after the first infestation and 30 days after the last infestation. Nineteen specimens were recovered from the first 5 feet of the duodenum, 34 from the rumen, and 1 from the pylorus (Table 17). Those from the duodenum were much more uniform in size than those from the duodenum of Calf III (Table 16). The smallest specimen measured 1.95 by 0.78 mm and the largest 2.86 by 0.99 mm. The average size of the 19 from the duodenum was 2.32 by 0.75 mm, while the average size of the 34 from

the rumen was 2.33 by 0.83 mm. The one specimen from the pylorus measured 2.43 by 0.81 mm.

The small number of parasites present in this host and their uniformity in size indicates that probably only one group of metacercariae

TABLE 18.—DATA ON SIZE (IN MILLIMETERS) OF *Cotylophoron cotylophorum* IN THE DUODENUM AND RUMEN OF NATURALLY INFESTED HOSTS

No.	Age of host: 6 months (16 present in duodenum) (7 present in rumen)		Age of host: 6 months (7 present in duodenum) (39 present in rumen)		Age of host: 2 years (none present in duodenum) (128 present in rumen)
	Duodenum	Rumen	Duodenum	Rumen (7 smallest)	Rumen (20 smallest)
1.....	1.04 x 0.41	2.54 x 1.17	0.96 x 0.83	2.44 x 1.19	1.82 x 1.22
2.....	1.22 x 0.52	2.62 x 1.30	1.35 x 0.78	2.60 x 1.30	1.87 x 1.08
3.....	1.97 x 0.80	2.63 x 1.04	1.43 x 0.85	2.62 x 1.56	1.92 x 1.04
4.....	2.00 x 0.91	2.70 x 0.98	1.56 x 0.91	2.71 x 1.56	1.92 x 1.17
5.....	2.10 x 0.96	2.99 x 1.22	1.87 x 1.06	2.83 x 1.53	2.00 x 1.06
6.....	2.15 x 0.85	3.12 x 1.30	2.08 x 0.98	3.12 x 1.45	2.00 x 1.17
7.....	2.21 x 0.65	3.30 x 1.01	2.39 x 0.78	3.12 x 1.58	2.02 x 1.17
8.....	2.23 x 0.78	2.05 x 1.17
9.....	2.26 x 0.70	2.08 x 1.04
10.....	2.26 x 0.72	2.08 x 1.09
11.....	2.26 x 0.78	2.08 x 1.11
12.....	2.34 x 0.78	2.08 x 1.17
13.....	2.36 x 0.98	2.08 x 1.17
14.....	2.52 x 0.98	2.15 x 1.11
15.....	2.62 x 0.98	2.21 x 1.17
16.....	2.70 x 0.91	2.21 x 1.30
17.....	2.26 x 1.06
18.....	2.34 x 1.17
19.....	2.47 x 1.22
20.....	2.52 x 1.30
Average	2.15 x 0.72	2.86 x 1.15	1.66 x 0.88	2.78 x 1.45	2.10 x 1.14

fed to the calf produced the infestation. The average size of worms from the duodenum of Calf IV is only slightly greater than that of the worms from the duodenum of Calf III, which seems to indicate that it was the last feeding of metacercariae to Calf IV which produced the infestation. A comparison of the average size of the parasites from the duodenum and the rumen of Calf IV clearly indicates that the worms were migrating and that very little growth occurs during their passage through the other parts of the stomach. That this passage is rapid is indicated by the fact that only one specimen was found in other parts of the stomach. The variability of the size of the worms in the rumen combined with the fact that there is a decided overlapping of size with those in the duodenum indicates that the worms migrate singly and that the

migratory period for any group of worms of the same age may extend over several days. Thus in Calf III, only 4 of 203 worms had migrated at the end of 21 days, and in Calf IV, 34 of 54 had migrated at the end of 30 days.

The size at which the worms migrated from the duodenum to the rumen in the experimental animals can be correlated with findings in naturally infested hosts. Many naturally infested animals were examined which contained the parasites in both the duodenum and the rumen. Others were examined in which only the duodenum or rumen was infested. Three such cases are presented in Table 18. The host represented in the first column was infested by 128 specimens in the rumen, 15 of which were mature. The average size of 20 of the smallest specimens is 2.1 by 1.14 mm. These worms are slightly shorter but wider than those from the rumen of experimental animals.

The parasites shown in column 2 of Table 18 were collected from the duodenum and rumen of a 6 months old calf. Sixteen were found in the duodenum which had an average size of 2.15 by 0.79 mm. Only 7 were found in the rumen. These averaged 2.86 by 1.15 mm. The parasites shown in column 3 were collected from another 6 months old calf. In the duodenum of this calf there were 7 parasites which averaged 1.66 by 0.88 mm. There were 39 specimens in the rumen, the largest of which measured 5.33 by 1.82 mm. The average size of 7 of the smallest specimens was 2.78 by 1.45 mm.

In these three cases there is further evidence that the worms migrate from the duodenum into the rumen of the final host when considerably less than 3 mm in length. A graph made using the data on specimens from both experimentally and naturally infested animals demonstrates that the greater number of worms migrate at a size of 2.37 by 0.98 mm.

From the above data it is possible to conclude that the metacercariae become excysted in the duodenum where they develop for 3 to 5 weeks. Following this period they migrate to the rumen at an average size of 2.37 to 0.98 mm.

DEVELOPMENT

The metacercariae excyst in the upper part of the duodenum but some may be carried as far posterior as 6 feet, as has been previously pointed out. The distribution of the various sizes found in the duodenum of experimental hosts indicates that some migration may occur within the limits in which they were found. The young forms are very active and migration for considerable distances is probably a matter of a very short time.

In the duodenum they are found attached to the mucosa by a power-

fully developed acetabulum, elongating and weaving their bodies from side to side. The worms are capable of moving rapidly from one position to another in the measuring worm manner. The color of the worm is reddish, which makes it very inconspicuous against the background of mucosa. To collect the worms, sections of the duodenum were placed in warm physiological salt solution. This increases their activity which makes them more easily seen. They were then scraped off and shaken free of all tissue from the duodenum.

The shape of the young worm is very much like that of the adult, being attenuated at the anterior end and widest in the testicular region. The dorsal surface is convex and the ventral surface is slightly concave. In cross section the body is nearly ovoid or round. The acetabulum is subterminal in living specimens, but when allowed to die unfixed the acetabulum opens posteriorly and the body becomes much flatter. The young worms are more active than the adults and are able to extend the body three times their contracted length while the adults are not capable of extending the body more than one and a half times their contracted length. Young individuals are also much more resistant than the adults. Some of the young specimens from the duodenum remain alive for 24 hours in cold physiological salt solution while older worms remain alive only 6 to 8 hours under similar conditions.

The age and size at which these parasites become mature was not determined experimentally, but by examining naturally infested animals a series of developmental stages varying from 1 to 11 mm in length was obtained. The smallest mature specimens measure 2.86 by 1.22 mm. Many are mature at a length of 3 mm. Krull (1934) has shown that these worms reach maturity in approximately three and one-half months, and the correctness of his findings has been pointed out previously (page 82). Since the worms migrate at an average size of 2.37 by 0.98 mm during the fourth and fifth weeks after infestation of the host and mature at the sizes given above it is evident that the rate of growth in the rumen is slow. This is shown also by the average size of the worms from the rumen of Calf I. Those worms which were 6 or 7 months old averaged only 3.55 by 2.32 mm.

The results obtained from the examination of a bull brought in to the animal pathology department of Louisiana State University are also of value in demonstrating the slow growth rate of these forms. This bull was brought in for observation on August 10, 1933, and was kept, as were the experimental calves, with no chance of becoming infested with *C. cotylophorum*. Upon examination on June 12, 1934, 26 specimens of *C. cotylophorum* were found in the rumen. The smallest of these worms measured 5.2 by 2.1 mm and the largest 7.0 by 2.75 mm. The average

size of the 26 was 5.6 by 2.34 mm. This bull had been confined slightly more than 10 months so that the smallest of the specimens must necessarily have been somewhat over 10 months of age. The largest specimens found from other naturally infested hosts never exceeded 11 by 3 mm. Specimens of this size were probably well over one year in age and represent the maximum size attained by the parasites in this host. No information other than the above was obtained concerning the longevity of these forms.

The adult of this species has been fully described by Fiscoeder (1901, 1903), Stiles and Goldberger (1910), Maplestone (1923), Bennett (1928), and Stunkard (1929) so that no detailed descriptions of structures will be given here. Descriptions of the development of structures of diagnostic importance and of structures which have not been described for this parasite are included.

Digestive Tract.—In the smallest individuals the digestive tract is identical in appearance to that of the largest worms. The caeca are in the same position in both, that is, in the dorso-lateral part of the body and terminate dorsal to the acetabulum. The posterior ends may be curved ventrally anterior to the acetabulum, but such variations are of no importance as pointed out by Maplestone (1923) and can be explained on the basis of differences in the degree of contraction.

The oral sucker changes consist of an increase in size only. Its growth, however, is not proportionate to that of the body. Its length in 1 mm worms as compared with the body length is in the ratio of about 1:5; in 2 to 3 mm worms the ratio is 1:6 or 1:7; and in 4 to 5 mm worms the ratio is 1:6. The oral sucker attains its maximum size in worms of 6 to 7 mm and the ratio is about 1:9. In a well extended specimen measuring 6 by 2.5 mm the oral sucker is 0.74 mm long, 0.58 mm wide and 0.46 mm dorso-ventrally. The worms may reach a size of 11 by 3 mm but there is no further increase in the size of the sucker.

The esophagus is the only structure of the digestive tract which possesses characteristics of specific importance. These are its length and the gradual increase in thickness of its walls from its anterior to its posterior end. The length of the esophagus is subject to considerable variation because of contraction but it increases in length as the worms develop, reaching its maximum length in worms of 6 to 7 mm in length as does the oral sucker. In the smallest worms obtained the esophagus is about 0.3 mm long and it increases steadily as the worms grow, but it never exceeds 0.9 mm in length. It bifurcates to form the intestinal caeca in the region of the genital pore in worms under 7 mm long (Figs. 81, 82, 83). However, the worms reach 11 mm in length and as a result the genital pore becomes distinctly post-bifurcal in position.

Stiles and Goldberger (1910:72) state that the genital pore of *C. indicum* is decidedly post-bifurcal and designate this as one of the differences between *C. indicum* and *C. cotylophorum*. Maplestone (1923:152) has pointed out that this characteristic is too variable to be of diagnostic importance for these two species, and the present findings support his contention.

The muscular thickening of the esophagus in *C. cotylophorum* is evident in the cercaria and becomes more evident as the worm develops in the final host. In the small worms the muscle wall at the proximal end of the esophagus is about 5 μ thick and at its distal end is 15 μ thick, while the diameter of the esophagus at the proximal end including the esophageal glands is 37 μ and at the distal end is 85 μ . The ratio of these measurements is found to vary from 1:2 in young worms to 1:4 in old worms. The thickness of the muscle wall at the proximal end does not exceed 20 μ , and it does not exceed 60 μ at the distal end. In the largest of the worms the total diameter of the proximal end does not exceed 0.17 mm and does not exceed 0.32 at the distal end. All of these measurements are subject to considerable variation but there is a normal increase in the size of the esophagus concurrent with growth. However, growth of the esophagus stops when the worms reach a size of 6 or 7 mm.

The difference in the thickness of walls of the esophagus at the two ends is very evident at all ages (Figs. 70-73), and the appearance of this structure is very similar to that in *C. cotylophorum* as described by Fiscoeder (1903:547) and Maplestone (1923-152).

Maplestone (p. 152) attempts to show that the esophagus of *C. indicum* and *C. cotylophorum* are identical, but I cannot agree with him. As stated, the esophageal thickening in the present material is very evident at all ages and sizes of worms. Stiles' and Goldberger's material possessed no such thickening, although their figure (1910:fig. 45, p. 66) shows that the walls of the esophagus are thick. The apparent muscular thickening shown in their figure is due to an increase in the lumen rather than to an increase of thickness of the walls. I have been able to determine this point from a sectioned specimen of a worm identified as *C. indicum* by these writers. The thickness of the walls of the esophagus in this material does not exceed 10 μ at its proximal end and does not exceed 20 μ at its distal end. The diameters of the two ends are 0.105 and 0.195 mm respectively. The above worm, which was not mature, measured 5.16 by 1.68 mm. These differences are too great to be the result of normal variation, as determined by a comparison with the present material.

Development of Sex Organs.—The primordia of the genital organs

are present in the cercaria, as previously described. In the smallest worms recovered from the final host the ovary, Mehlis' gland, and testes were differentiated (Fig. 77). The ovary and Mehlis' gland are represented by two small masses of cells located near the center of the body above the anterior margin of the acetabulum. From Mehlis' gland a cord of cells, in which there is no lumen, passes ventrally over the anterior margin of the acetabulum to the ventral region of the body. It turns forward and continues anteriorly near the ventral surface to reach a mass of cells located above the position of the future genital pore. The testes are located laterally, one on each side of the cord immediately anterior to the acetabulum. Their method of formation was not determined, but their position suggests that they are set off from the ventral side of the mass of cells located anterior and dorsal to the acetabulum in the cercaria. The ovary and Mehlis' gland doubtless are formed from the dorsal part of this mass. There is no indication of a lumen in the cord of cells at any place. The testes are surrounded by a thin membrane but no vasa efferentia were observed. The genital pore is not yet formed, and the only indication of a genital sucker is a slight thickening of the body wall and a mass of deeply-staining cells which are probably cells of the prostate gland.

The degree of development described above is reached in worms of approximately 1.0 by 0.65 mm. In individuals of this size the ovary measures 0.045 by 0.03 mm, Mehlis' gland 0.037 by 0.022 mm, the testes 0.065 by 0.045 mm, the diameter of the cord of cells 0.045 mm, and the mass of cells surrounding the genital pore 0.097 by 0.032 mm.

The vasa efferentia, the vas deferens, the uterus, and a genital pore were clearly distinguished in an individual 1.17 by 0.8 mm. The uterus follows a course from Mehlis' gland to the ventral side of the body similar to that of the cord of cells described in the above form and is doubtless formed from it. Here it turns dorsally and anteriorly until it reaches the center of the body. It passes forward for a short distance and then turns ventrally. Near the ventral surface it turns forward to the genital pore. It joins the vas deferens, and the common duct thus formed, the ductus hermaphroditicus, opens to the outside (Fig. 63).

In this same specimen the testes have taken the tandem arrangement characteristic of the mature worm. The vasa efferentia are conspicuous and are easily traced. The one from the posterior testis passes anteriorly and dorsally until it reaches the mesial side of the right caecum where it turns forward. The vas efferens from the anterior testis passes forward mesial to the left caecum. The two unite in the center of the body a short distance posterior to the genital pore and immediately anterior to the descending uterus. The vas deferens thus formed coils ventrally and

anteriorly to the genital pore. It is located dorsal to the uterus and unites with it to form the ductus hermaphroditicus.

The position of the vasa efferentia and vas deferens indicates that these structures are formed from the dorsal part of the cord of cells described in the smaller individuals. The ventral position of the uterus near the genital pore indicates that it is derived from the ventral part of the cord. The descent of the uterus posterior to the loop formed by the union of the vasa efferentia supports this conclusion also.

The genital sucker becomes more evident in specimens of this size, although as yet there are no definitely differentiated muscles in it. It consists of a compact mass of cells surrounding the terminations of the vas deferens, the uterus, and the ductus hermaphroditicus. This mass of cells is approximately 0.12 mm long, 0.108 mm wide, and 0.072 mm thick (Fig. 63).

All of the male and female genital structures, with the exception of the vitellaria, are formed before the worms migrate from the duodenum to the rumen.

Following the stage just described the other structures characteristic of the genitalia of the mature worm are rapidly developed, being present in worms 2.5 mm long and less than 3 weeks old (Fig. 76). The vas deferens is differentiated into several distinct regions: a seminal vesicle, a pars muscosa, the prostate gland, and the ductus ejaculatorius, which joins the ductus hermaphroditicus. The ductus hermaphroditicus passes through the center of a minute hermaphroditic papilla which opens into a small genital atrium in the genital papilla. The copulatory structures and the terminations of the male and female ducts are enclosed in the genital sucker.

Laurer's canal is well developed in individuals of this size also. It passes from the oviduct dorsally and laterally to open to the exterior behind the excretory pore and to the left of the median line. The only other development in the female system is the formation of the metraterm.

There is no recognizable change in the worms immediately following their migration into the rumen, which is a second indication that the time required for migration is very short. The fact that only one worm was found in other parts of the stomach other than the rumen has been considered above as an indication that the worms migrate from the duodenum to the rumen rather quickly.

No worms of known age were studied from the time of migration but many specimens representing all sizes and stages of development were secured from naturally infested hosts. There is very little increase in size before the worms reach sexual maturity and this increase is in

diameter. The smallest mature worm collected measured 2.86 by 1.43 mm. The most conspicuous changes in the small mature worms as compared to the immature ones are the increase in size of the genital organs and the development of the vitellaria (cf. Figs. 76 and 79). In the smallest of the mature worms the vitellaria are very sparsely developed in the lateral regions of the body and extend from the esophagus to the acetabulum. The testes in immature worms at the time of migration are round and smooth and measure only 0.1 mm in diameter. In the smallest of the mature worms the testes are 0.36 by 0.26 mm; they extend dorso-ventrally for a distance of 0.48 mm and are distinctly lobed. There is an increase in the size of the ovary from 0.075 mm to 0.196 mm in diameter. The uterus in both the immature and small mature worms is straight but its diameter increases from 0.032 mm in the immature to 0.081 mm in the mature worms. The vasa efferentia remain unchanged but the vas deferens becomes greatly coiled. The genital sucker and copulatory structures are also much more conspicuous in the small mature worms.

The genital sucker in worms which have just migrated to the rumen measures 0.17 mm in diameter and the muscle mass is about 0.14 mm thick (Fig. 76). In small mature worms this structure measures 0.4 mm in diameter and is 0.22 mm thick.

The only marked change which occurs after sexual maturity is attained is the rapid development of the vitellaria. In worms only 3.5 mm in length they have reached a state of development comparable to that in the largest worms (Fig. 80). They extend in closely grouped follicular masses from the oral sucker to the acetabulum, principally in the extra-caecal zones but approach the median line both dorsally and ventrally. The uterus becomes more coiled as larger numbers of eggs are produced and fills all available space between the acetabulum and the posterior testis. It is coiled transversely dorsal to the testes, and more coils are formed in the ventral region of the body anterior to the anterior testis.

Stunkard (1929:244) found that *C. cotylophorum* reached maturity at a much smaller size in calves than in antelopes, but he could not explain the incongruity. He did not give the size of the mature worms from the calves but specimens as long as 6 mm from the antelopes were not mature. These findings clearly indicate that these parasites mature at a much smaller size in some hosts than in others.

The genital organs are much larger in fully grown worms than in those just reaching maturity (cf. Figs. 79 and 81). The testes are located in tandem arrangement near the center of the body and occupy approximately two-fifths of the body length. The seminal vesicle is

greatly expanded and coiled; the pars muscosa is thick, muscular-walled, and coiled; the pars prostatica located directly above the genital pore is straight and measures about 0.20 by 0.22 mm. Its lumen is large but narrows abruptly as it becomes continuous with the ductus ejaculatorius. The ductus ejaculatorius is about 0.19 mm long and opens into the ductus hermaphroditicus. The hermaphroditic papilla varies in length with its state of contraction but its length is about 0.1 mm. The genital papilla encloses a small atrium into which the protrusible hermaphroditic papilla projects. The walls of the genital papilla are very muscular and are about 50 μ thick. The genital papilla when protruded measures about 0.19 by 0.14 mm and projects into the cavity of the genital sucker (Fig. 74).

The ovary and Mehlis' gland in fully grown worms are located above the anterior margin of the acetabulum. The uterus is crowded with eggs and is coiled anterior to the acetabulum, dorsal to the testes, and in the ventral region of the body anterior to the anterior testis. The muscular metraterm joins the ductus hermaphroditicus. The union of the male and female ducts forms a distinct but small vesicle at the inner end of the ductus hermaphroditicus.

The genital sucker consists of a muscular mass which encloses the terminal ends of the male ducts and the copulatory apparatus. This structure increases in size as the worms develop but does not exceed 0.7 mm in diameter in any of the present material. Its walls are approximately 0.3 mm thick. The cavity of the sucker is considered as a genital atrium by Fischöder (1903) and Maplestone (1923). The genital pore is the opening of the sucker while the pore of the ductus hermaphroditicus is the porus hermaphroditicus.

When the worms are emitting eggs or sperm the genital papilla can be protruded for a short distance beyond the edge of the genital sucker. At the same time rapid contractions and relaxations of the muscles of the genital sucker occur.

The genital atrium, the genital papilla, and the genital sucker are subject to considerable change in shape and size. The hermaphroditic papilla is also subject to great changes in size and appearance. However, in the present material after their development was completed these structures were evident in all ages and sizes of worms, and in all states of contraction (Figs. 68, 69, 74, 75).

Maplestone (1923:153-155, figs. 8, 9) describes in detail the extreme variability in the appearance of the genital sucker and copulatory apparatus of *C. cotylophorum* and concludes that these structures are of no diagnostic value. His figures (Figs. 8, A1, A2, B) represent the appearance of the genital sucker and copulatory apparatus of *C.*

cotylophorum as described by Fiscoeder (1903:548, fig. 38) and of *C. indicum* as described by Stiles and Goldberger (1910:69, fig. 48). As a result of his observations on these structures and on the variability of the esophagus of *C. cotylophorum* he considers *C. indicum* as a synonym of *C. cotylophorum*.

Fukui (1929:319) agrees with Maplestone and designates *C. indicum* as a synonym of *C. cotylophorum*.

As originally described by Fiscoeder the genital sucker of *C. cotylophorum* is much larger and much more distinctly set off from the body parenchyma than in Maplestone's or the present material (Fig. 67). Fiscoeder does not describe a genital papilla in *C. cotylophorum* and the male and female ducts remain separate in the hermaphroditic papilla.

The genital sucker of *C. indicum* as described by Stiles and Goldberger is also distinctly set off from the body parenchyma and they do not describe a genital papilla as being present. In the present study some of the original material of Stiles and Goldberger was studied and no genital papilla was found. There is also a distinct difference in the appearance of the genital sucker of *C. indicum* and that of *C. cotylophorum* as described and figured by Maplestone (1923:156, fig. 9) and as described in this paper.

Stunkard (1923:138) believes that Maplestone's conclusions as to the importance of these structures are erroneous. I am of the same opinion, and in view of the decided differences in the appearance of the genital sucker, the copulatory apparatus, and esophagus of *C. indicum* as compared to *C. cotylophorum* I cannot consider these two species as synonymous.

The present specimens of *C. cotylophorum* agree in every detail with Maplestone's description of this species with the exception of the extreme variability of the copulatory apparatus as pointed out above, with Stunkard's description (1929:244-251), and with specimens loaned me by Krull. However, I believe that the differences between the genital sucker and copulatory apparatus of Fiscoeder's material and the present material are of diagnostic importance. Since these differences are the only ones which have been observed I am hesitant in considering these differences as being of specific value in view of Maplestone's and Fukui's findings.

Excretory System.—The arrangement of the excretory system in the young and mature specimens is very similar to that of the cercaria (Fig. 60). The details of the system were not studied. Only living immature specimens under pressure were studied. It was possible in this way to determine the course and extent of the larger ducts and the position of the bladder.

The bladder is an elongate structure located dorsally in the posterior region of the body, extending from near the posterior margin of the acetabulum to slightly past the anterior margin. It opens to the exterior through a narrow short muscular duct lined with cuticula continuous with that of the body surface. It may pass directly to the surface from the middle or anterior end of the bladder or may extend forward from it (Figs. 77, 78). In young specimens the former condition is more often found, and it is only in the more fully developed and very extended individuals that the duct opens very far in front of the bladder. The pore is located in the medial dorsal line, usually directly above the posterior margin of the posterior testis in mature specimens, but it may be as far forward as the middle of the anterior testis, or as far posterior as the anterior margin of the acetabulum. Its position relative to these organs depends entirely on the age of the individual and its state of contraction. However, the position of the pore may be considered as pre-vesicular, being dorsal to the bladder only in immature or contracted mature specimens. Maplestone (1923:157, text-fig. 11) has described and shown similar conditions in specimens of *C. cotylophorum* of different ages. I did not observe the pore to be post-vesicular in any of my material as Maplestone has figured it in a very young specimen. Fukui (1929:275) has described similar variations in *Paramphistomum explanatum*, *P. cervi*, and *P. orthocoelium*. He states that the pore is very variable in position and cannot be used for exact diagnostic purpose, but that it is roughly definite for species.

The main excretory canals are located the same as in the cercaria. From their union with the postero-lateral angle of the bladder on each side they pass outward and forward. Immediately posterior to the middle of the body length these canals bend mesially and a cross connection passes across the middle line and sends off a forward diverticulum. The main canals then curve outward and forward. The diverticulum present just posterior to the eye in the cercaria is present in these older worms, and in them it receives a small duct which in turn receives branches from the esophageal region. The main canals continue forward from this diverticulum until they reach the posterior margin of the oral sucker. Here they turn abruptly on themselves and pass posteriorly in the lateral regions of the body. These posterior extensions could not be traced to their terminations but doubtless they extend as far back as the acetabulum, as they do in the cercaria. A small duct on each side extends forward from the turning point of the main canals which drains the anterior region of the body. Numerous small ducts which are symmetrically located empty into the larger canals throughout their course.

The anterior diverticulum from the cross connection becomes greatly enlarged and it also receives small ducts from the anterior dorsal region of the body.

A detailed description of the excretory system was made from preserved material by Bennett (1928:22-23).

The excretory system of this material is very similar to that of *Gastrothylax* and *Paramphistomum* as described by Fukui (1929:272). This type of system he designates as Type A and calls it H-shaped.

SPECIFIC DESCRIPTION OF *Cotylophoron cotylophorum*

The following specific description of *C. cotylophorum* is based entirely on the characteristics of the material used in the present study.

Body of mature worm 3 to 11 mm long by 1.15 to 3 mm wide; conical in form, greatest width in testicular region; tapers to bluntly pointed anterior end, posterior end broadly rounded; dorsal surface convex longitudinally and transversely, ventral surface concave longitudinally, convex transversely; oval to round in cross section. Surface without spines or papillae. Genital pore bifurcal or slightly post-bifurcal, at junction of first and second body thirds, surrounded by genital sucker 0.4 to 0.7 mm in diameter which forms a distinct projection in the median ventral line. Acetabulum at posterior end, distinctly subterminal 0.75 to 1.36 mm in diameter. Mouth at blunt anterior extremity; oral sucker pyriform in sagittal section; 0.52 mm long, 0.45 mm wide and 0.39 mm in dorso-ventral diameter in small mature worms, its maximum in fully grown individuals 0.74 mm long, 0.58 mm wide and 0.45 mm in dorso-ventral diameter; esophagus slightly longer than oral sucker; its walls increase in thickness posteriorly, ratio of thickness of anterior wall to posterior wall 1.3; caeca arise from dorso-lateral aspects of end of esophagus, terminate in acetabular zone. Excretory pore in median dorsal line about at junction of median and posterior body thirds; excretory bladder extends posteriorly from pore above acetabulum; lateral excretory tubes extend from ventral and postero-lateral margin of bladder to oral sucker, turn sharply posterior to acetabulum; cross connection between lateral ducts in dorsal region and near middle of body length, median diverticulum extends forward for short distance from cross connection, lateral diverticulum from each lateral duct a short distance posterior to oral sucker.

Testes large, lobate, about size of oral sucker in young mature worms, larger than acetabulum in old specimens, in median line, tandem arrangement; union of vasa efferentia slightly anterior to anterior testis; vas deferens coiled; its vesicula seminalis coiled, expanded; pars musculosa coiled, narrow; pars prostatica straight, located directly above genital

sucker; ductus ejaculatorius short, unites with metraterm to form ductus hermaphroditicus; hermaphroditic papilla short, protrusible, arises from the vertex of a conspicuous genital papilla, almost filling the cavity of the papilla; genital papilla in turn surrounded by the genital sucker.

Ovary and Mehlis' gland above anterior margin of acetabulum; Laurer's canal passes over excretory bladder, opens posterior to excretory pore and left of median dorsal line; uterus coiled anterior to acetabulum, passes anteriorly dorsal to testis, descends vertically over the anterior margin of anterior testis, anteriorly again ventral to vas deferens, enters genital sucker; and metraterm unites with ductus ejaculatorius.

SUMMARY AND CONCLUSIONS

Cotylophoron cotylophorum is a widely distributed parasite of ruminants but has not been previously reported from the mainland of North America.

The time required for the miracidium to develop varies directly with temperature. In the present experiments eggs kept at room temperatures hatched in 11 to 29 days.

The structures of the miracidium develop in sequence and are recognizable before hatching occurs.

The miracidium is similar to other amphistome miracidia. A study of the descriptions of 18 different species of miracidia indicates that the number and arrangement of ciliated epidermal cells is of taxonomic value.

The snails *Fossaria parva* and *F. modicella* are capable of serving as the intermediate hosts of *C. cotylophorum*. The former is the natural host of this parasite in Louisiana.

The miracidium penetrates the mantle, head, and foot of the snail, loses its ciliated epidermal cells, and transforms into a sporocyst.

The sporocyst develops rapidly and produces 9 rediae.

The rediae are born at an average size of 0.188 by 0.056 mm and migrate into the liver and ovo-testis where their development is complete. Each redia produces approximately 25 cercariae.

Mother rediae may occur in the life cycle but were observed in only one instance.

Cercariae are born in an undeveloped condition and continue their development in the liver and ovo-testis.

The time required for the development of the sporocyst, redia, and cercaria varies directly with temperature. Infested snails kept under natural temperature conditions shed cercariae in 30 to 91 days.

The cercariae encyst on vegetation and the metacercaria lives for over 3 months.

The metacercariae become excysted in the duodenum of the final host. Migration from the duodenum to the rumen begins in 21 days at an average size of 2.37 by 0.98 mm and may continue over a period of about 14 days. The worms do not migrate at the same age or size.

The worms become mature after reaching the rumen at an age of about three and a half months, at a size of approximately 3.0 by 1.15 mm. They reach their maximum size in about one year.

The time required to complete the life cycle of *C. cotylophorum* varies from about 5 to 8 months.

C. indicum is not a synonym of *C. cotylophorum*.

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EXPLANATION OF PLATES

All figures were made with the aid of a camera lucida with the exception of Fig. 80 which is a graphic reconstruction.

Abbreviations Used

<i>a</i>	acetabulum	<i>gc</i>	germ cell
<i>an</i>	anterior nerve	<i>gn</i>	gut nucleus
<i>ap</i>	apical papilla	<i>gp</i>	genital pore
<i>bm</i>	basement membrane	<i>g pa</i>	genital papilla
<i>bp</i>	birth pore	<i>gs</i>	genital sucker
<i>br</i>	brain	<i>gt</i>	germinal tissue
<i>c</i>	cuticula	<i>hp</i>	hermaphroditic papilla
<i>ca</i>	caecum	<i>i</i>	intestine
<i>ca p</i>	primordium of caecum	<i>La c</i>	Laurer's canal
<i>cc</i>	central cavity	<i>lc</i>	lens cell
<i>ceb</i>	caudal excretory bladder	<i>le</i>	lateral evagination
<i>ced</i>	caudal excretory duct	<i>led</i>	lateral excretory duct
<i>cep</i>	caudal excretory pore	<i>lm</i>	longitudinal muscle
<i>cer</i>	cercaria	<i>m</i>	metraterm
<i>cg</i>	cerebral ganglion	<i>me</i>	median evagination
<i>cm</i>	circular muscles	<i>Mg</i>	Mehlis' gland
<i>com</i>	commisure	<i>n</i>	nerve
<i>cu c</i>	cuticular cell	<i>nc</i>	nerve cell nucleus
<i>cy c</i>	cystogenous cell	<i>nf</i>	nerve fiber
<i>cyg</i>	cystogenous granule	<i>nu</i>	nucleus
<i>de</i>	ductus ejaculatorius	<i>op</i>	oral plug
<i>dh</i>	ductus hermaphroditicus	<i>os</i>	oral sucker
<i>eb</i>	excretory bladder	<i>ov</i>	ovary
<i>ec</i>	epithelial cell	<i>p</i>	plug
<i>ed</i>	excretory duct	<i>pe</i>	primitive epithelium
<i>em</i>	embryo	<i>pg</i>	penetration gland
<i>en</i>	eye nerve	<i>pgd</i>	penetration gland duct
<i>ep₁</i>	epidermal cell, row 1	<i>ph</i>	pharynx
<i>ep₂</i>	epidermal cell, row 2	<i>ph c</i>	pharyngeal cuticular cell
<i>ep₃</i>	epidermal cell, row 3	<i>pi</i>	pigment
<i>ep₄</i>	epidermal cell, row 4	<i>pm</i>	pars muscosa
<i>epn₁</i>	epidermal cell nucleus, row 1	<i>pp</i>	pars prostatica
<i>epn₂</i>	epidermal cell nucleus, row 2	<i>pn</i>	posterior nerve
<i>epn₃</i>	epidermal cell nucleus, row 3	<i>sg</i>	salivary gland
<i>epn₄</i>	epidermal cell nucleus, row 4	<i>sn</i>	subepithelial nucleus
<i>es</i>	esophagus	<i>sp</i>	sensory papilla
<i>es:</i>	esophageal cell	<i>sl</i>	sporocyst tissue
<i>el</i>	excretory tubule	<i>sv</i>	seminal vesicle
<i>exp</i>	excretory pore	<i>i</i>	tail
<i>ey</i>	eye	<i>te</i>	testis
<i>fc</i>	flame cell	<i>u</i>	uterus
<i>g</i>	gut	<i>v</i>	vitellaria
<i>ga</i>	genital atrium	<i>vd</i>	vas deferens
<i>gb</i>	germ ball	<i>ve</i>	vas efferens

PLATE I

FIGS. 1-11.—Developing miracidia. Scale 0.05 mm.

FIG. 12.—Four-cell stage in development of the miracidium.
Scale 0.03 mm.

FIG. 13.—Flame cell of miracidium. Scale 0.01 mm.

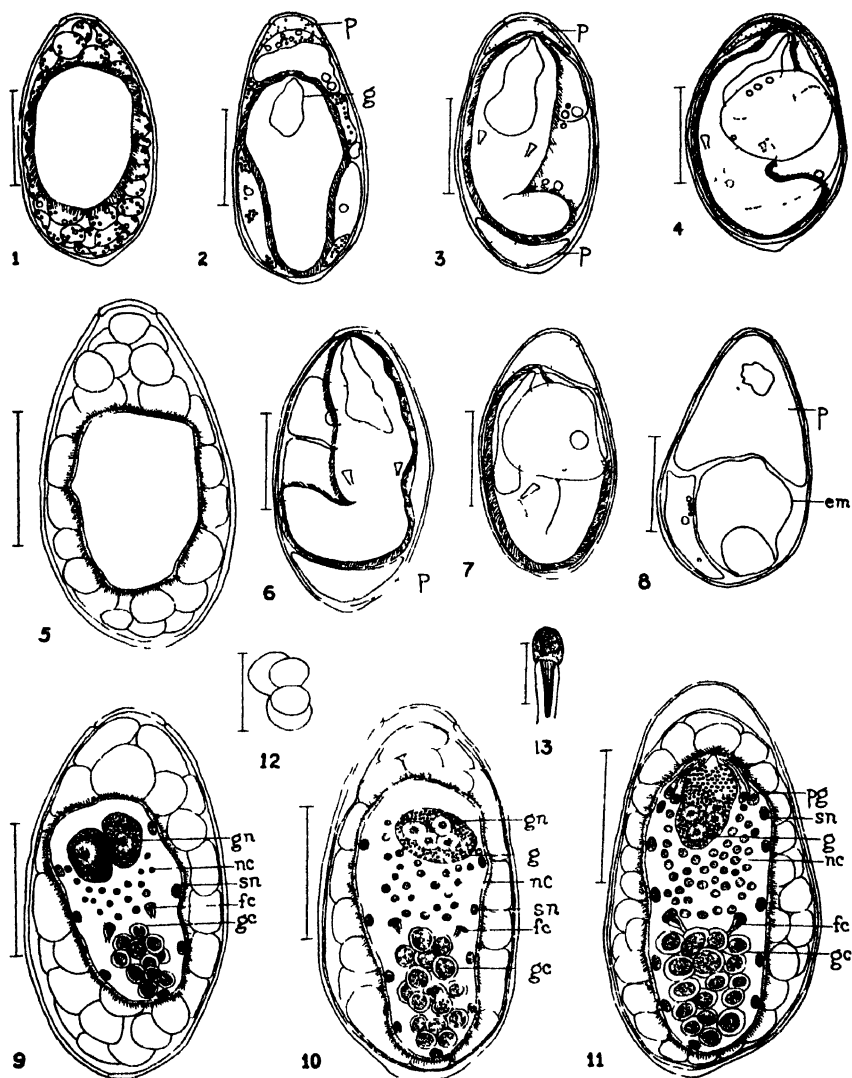


PLATE I

PLATE II

FIGS. 14-17.—Mature miracidia. Scale 0.05 mm.

FIGS. 18-21.—Cross sections of miracidia. Scale 0.02 mm.

FIG. 22.—Frontal section of anterior end of miracidium. Scale
0.02 mm.

FIG. 23.—Frontal section of miracidium. Scale 0.02 mm.

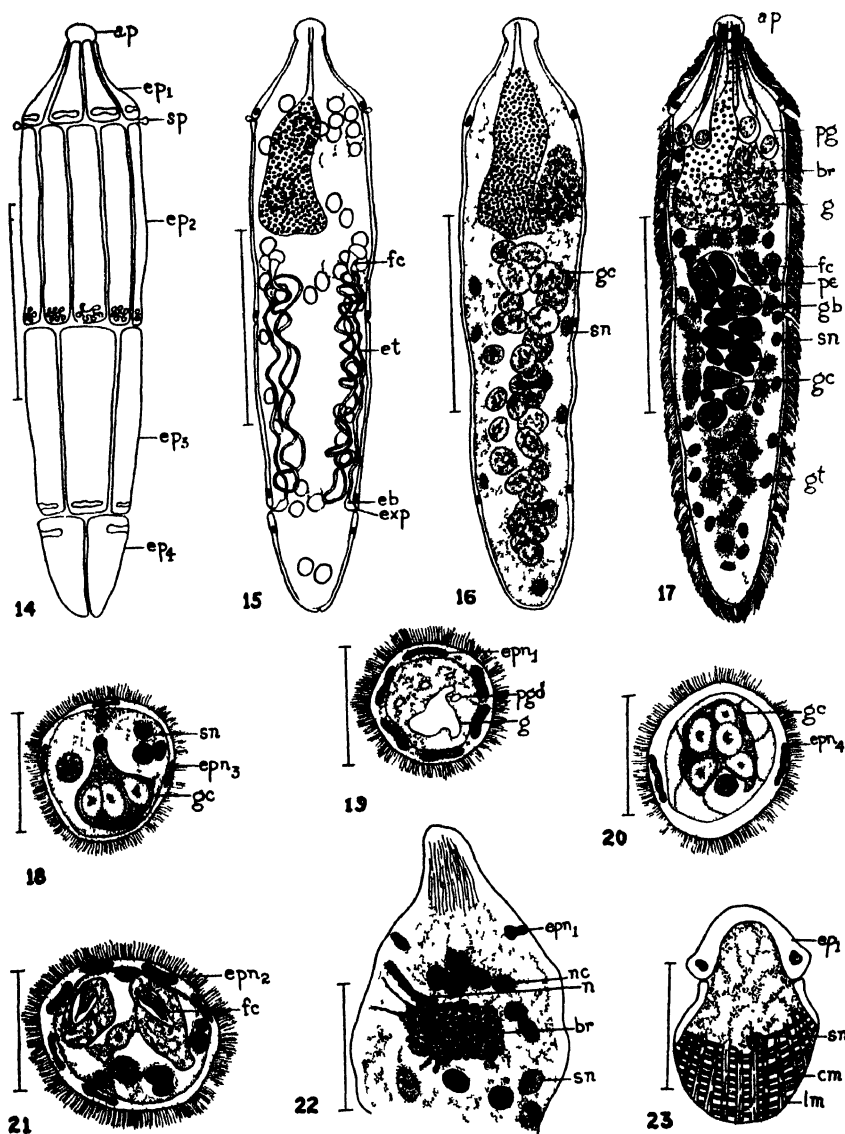


PLATE II

PLATE III

FIG. 24.—Miracidium in lymph duct of snail. Scale 0.03 mm.

FIG. 25.—Five-day sporocyst. Scale 0.05 mm.

FIG. 26.—Mature sporocyst. Scale 0.1 mm.

FIG. 27.—Germinal and epithelial cells in body wall of sporocyst. Scale 0.01 mm.

FIG. 28.—Twenty-four-hour sporocyst. Scale 0.03 mm.

FIG. 29.—Twelve-hour sporocyst. Scale 0.02 mm.

FIG. 30.—Longitudinal section of sporocyst. Scale 0.1 mm.

FIG. 31.—Longitudinal section of forty-eight-hour sporocyst.
Scale 0.03 mm.

FIG. 32.—Body wall of sporocyst. Scale 0.05 mm.

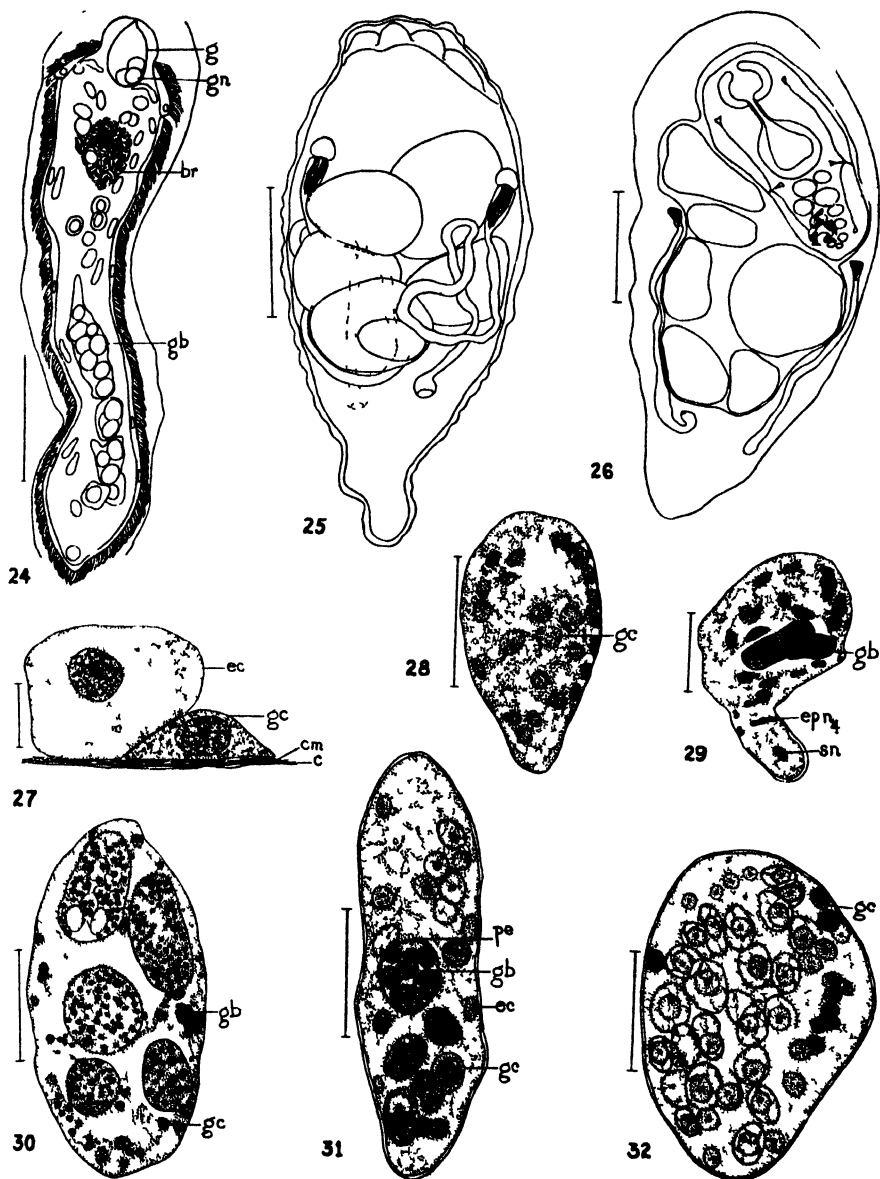


PLATE III

PLATE IV

FIG. 33.—Mature redia. Scale 0.3 mm.

FIG. 34.—Frontal section of redia showing salivary glands.
Scale 0.05 mm.

FIG. 35.—Mature sporocyst. Scale 0.1 mm.

FIG. 36.—Redia about to escape from sporocyst. Scale 0.05
mm.

FIG. 37.—Frontal section of redia showing brain. Scale 0.05
mm.

FIG. 38.—Sagittal section of anterior end of redia. Scale
0.1 mm.

FIG. 39.—Posterior end of redia showing germ cells and de-
veloping cercariae. Scale 0.1 mm.

FIG. 40.—Redia in pocket of sporocyst tissue. Scale 0.3 mm.

FIG. 41.—Posterior end of redia showing exhaustion of germ
cells. Scale 0.1 mm.

FIG. 42.—Longitudinal section of immature redia. Scale 0.03
mm.

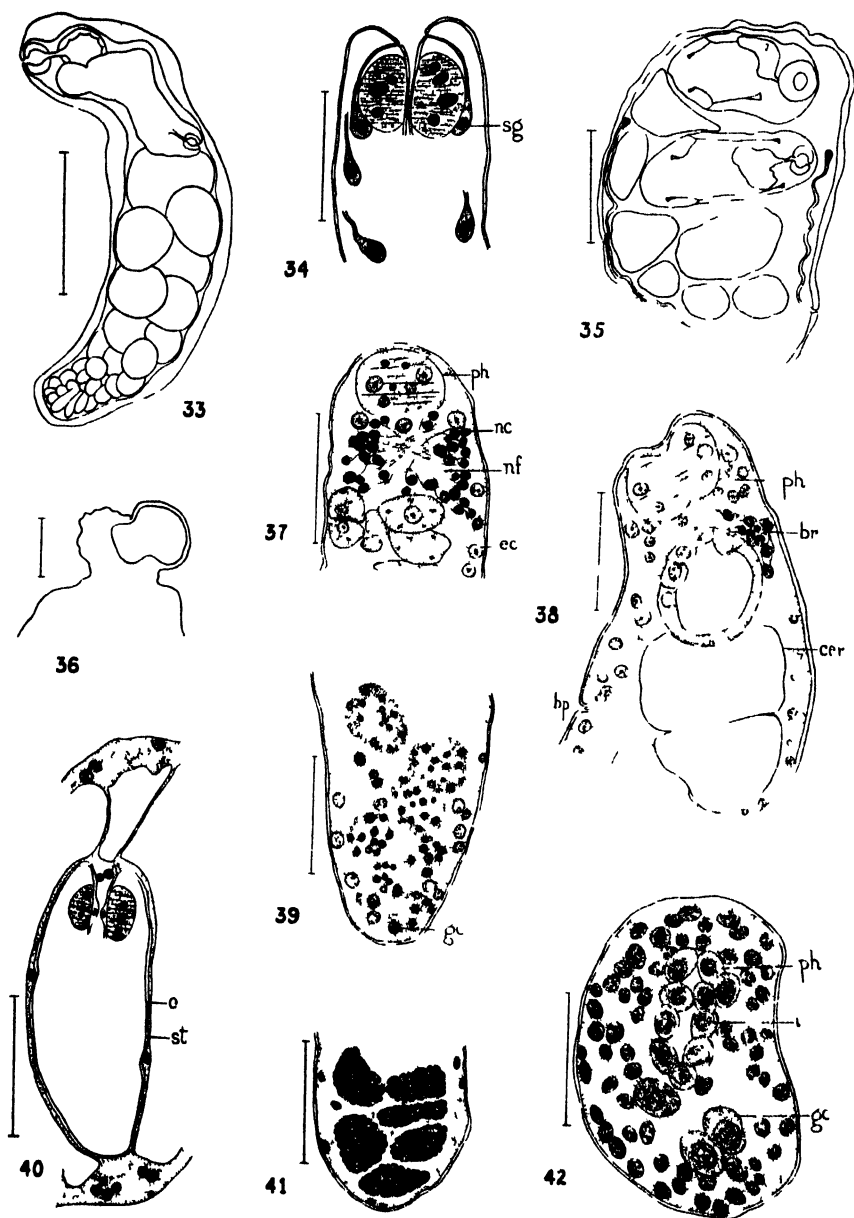


PLATE IV

PLATE V

FIGS. 43, 44.—Longitudinal section of anterior end of immature redia. Scale 0.03 mm.

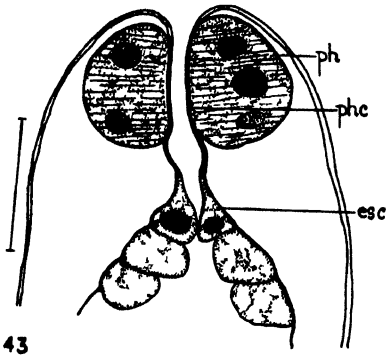
FIG. 45.—Lateral view of posterior end of cercaria. Scale 0.05 mm.

FIG. 46.—Mature redia. Scale 0.2 mm.

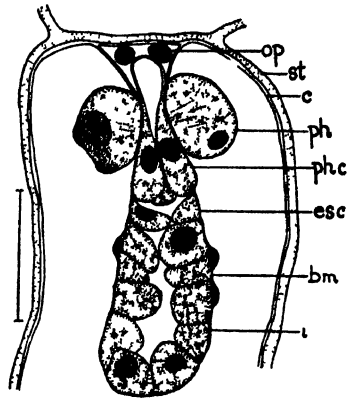
FIG. 47.—Oral sucker and esophagus of mature cercaria, sagittal section. Scale 0.02 mm.

FIG. 48.—Mature redia. Scale 0.2 mm.

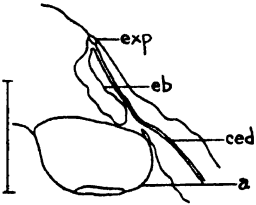
FIG. 49.—Longitudinal section of anterior end of immature redia. Scale 0.03 mm.



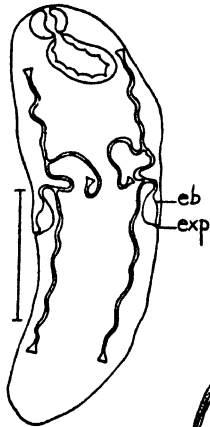
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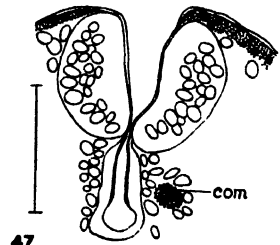
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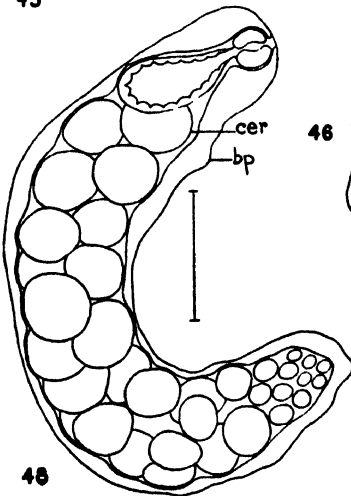
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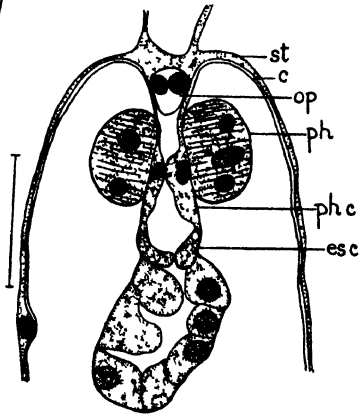
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PLATE V

PLATE VI

- FIG. 50.—Immature cercaria, dorsal view. Scale 0.05 mm.
- FIG. 51.—Mature cercaria, ventral view. Scale 0.1 mm.
- FIG. 52.—Immature cercaria, dorsal view. Scale 0.05 mm.
- FIG. 53.—Metacercaria, lateral view. Scale 0.01 mm.
- FIG. 54.—Cross section of tail of mature cercaria. Scale 0.04 mm.
- FIG. 55.—Frontal section of immature cercaria. Scale 0.05 mm.
- FIG. 56.—Immature cercaria, dorsal view. Scale 0.05 mm.
- FIG. 57.—Immature cercaria, ventral view. Scale 0.05 mm.
- FIG. 58.—Mature cercaria, sagittal section. Scale 0.05 mm.

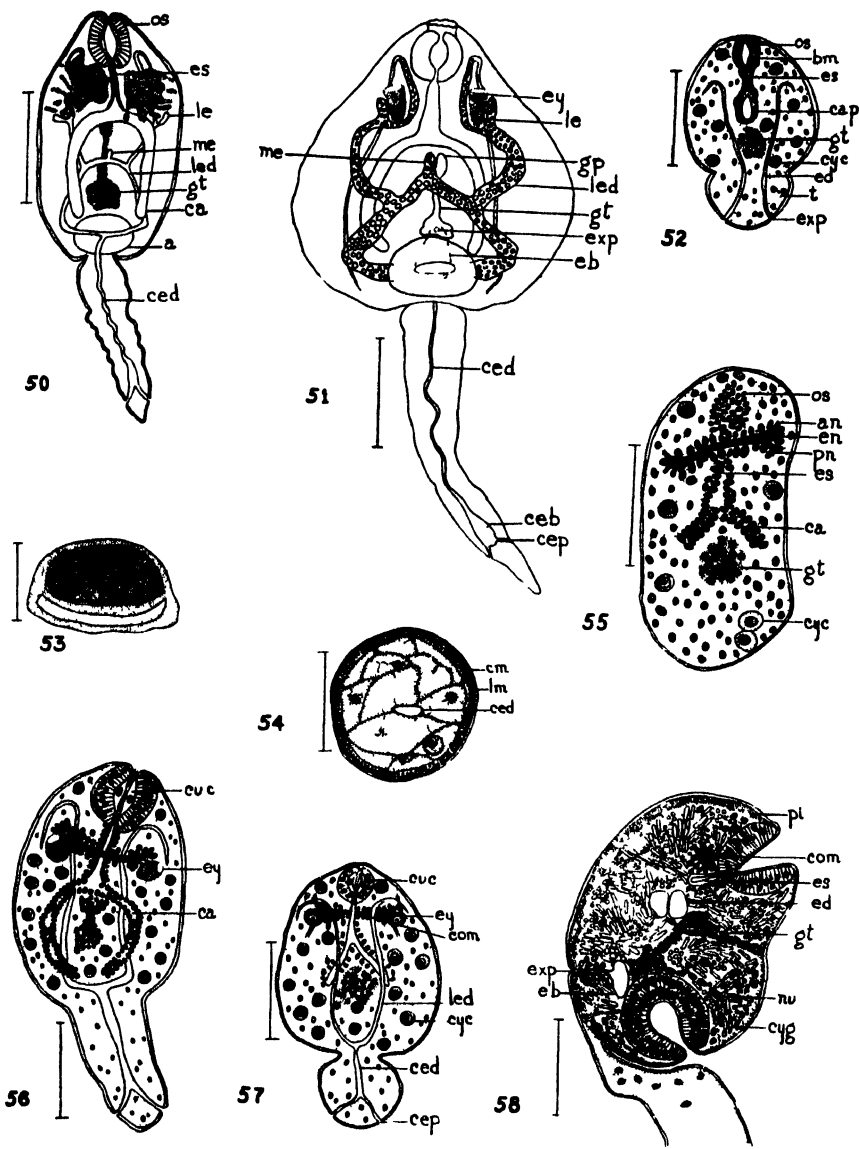


PLATE VI

PLATE VII

FIG. 59.—Immature cercaria, dorsal view showing pigment.
Scale 0.1 mm.

FIG. 60.—Excretory system, immature specimen. Scale 0.2 mm.

FIG. 61.—Immature cercaria, lateral view showing development of pigment. Scale 0.1 mm.

FIG. 62.—Section of developing eye. Scale 0.01 mm.

FIG. 63.—Cross section through genital sucker of a worm
1.17 x 0.84 mm. Scale 0.1 mm.

FIGS. 64, 65.—Sections of developing eye. Scale 0.02 mm.

FIG. 66.—Anterior end of mature cercaria, sagittal section.
Scale 0.04 mm.

FIG. 67.—Cross section through genital sucker of a worm
3.65 x 2.45 mm. Scale 0.5 mm.

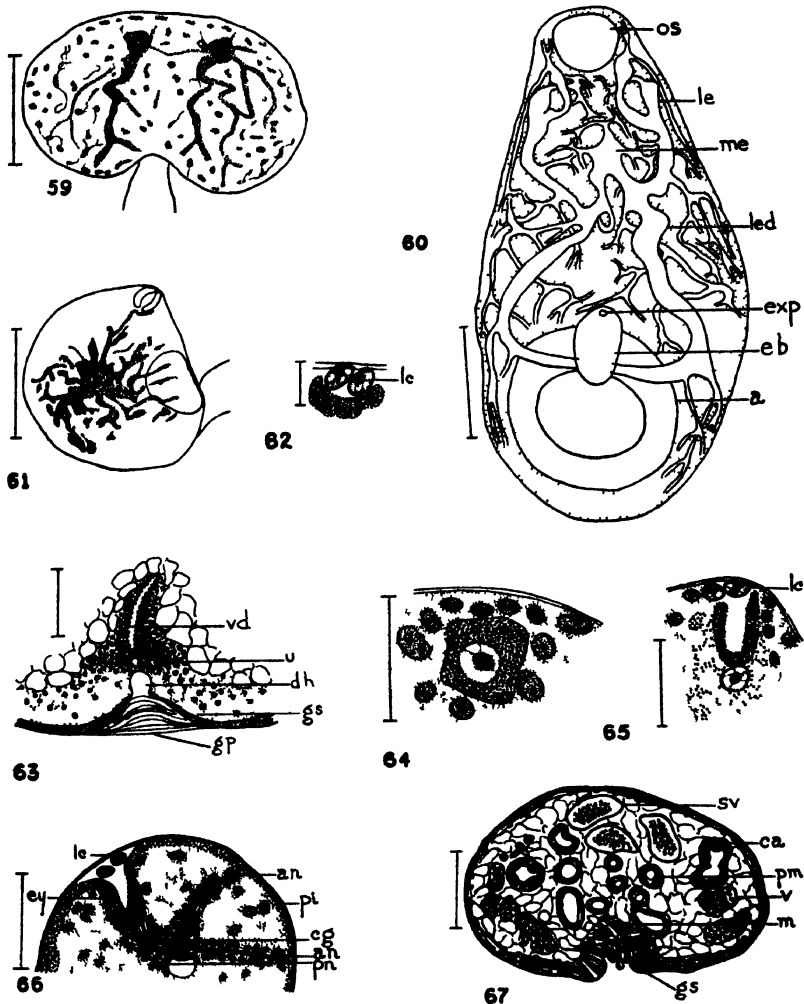


PLATE VII

PLATE VIII

- FIG. 68.—Sagittal section through genital complex of a specimen 2.5 x 0.72 mm. Scale 0.2 mm.
- FIG. 69.—Cross section through genital sucker of a specimen 2.95 x 1.13 mm. Scale 0.1 mm.
- FIG. 70.—Sagittal section of anterior end of a specimen 1.09 x 0.39 mm. Scale 0.2 mm.
- FIG. 71.—Sagittal section of anterior end of a specimen 2.46 x 0.63 mm. Scale 0.5 mm.
- FIG. 72.—Sagittal section of anterior end of a specimen 6.0 x 2.75 mm. Scale 1.0 mm.
- FIG. 73.—Sagittal section of anterior end of a specimen 9.0 x 2.75 mm. Scale 1.0 mm.
- FIG. 74.—Cross section through genital sucker of a specimen 8.0 x 2.75 mm. Scale 0.1 mm.
- FIG. 75.—Cross section through genital sucker of a specimen 4.0 x 1.15 mm. Scale 0.1 mm.

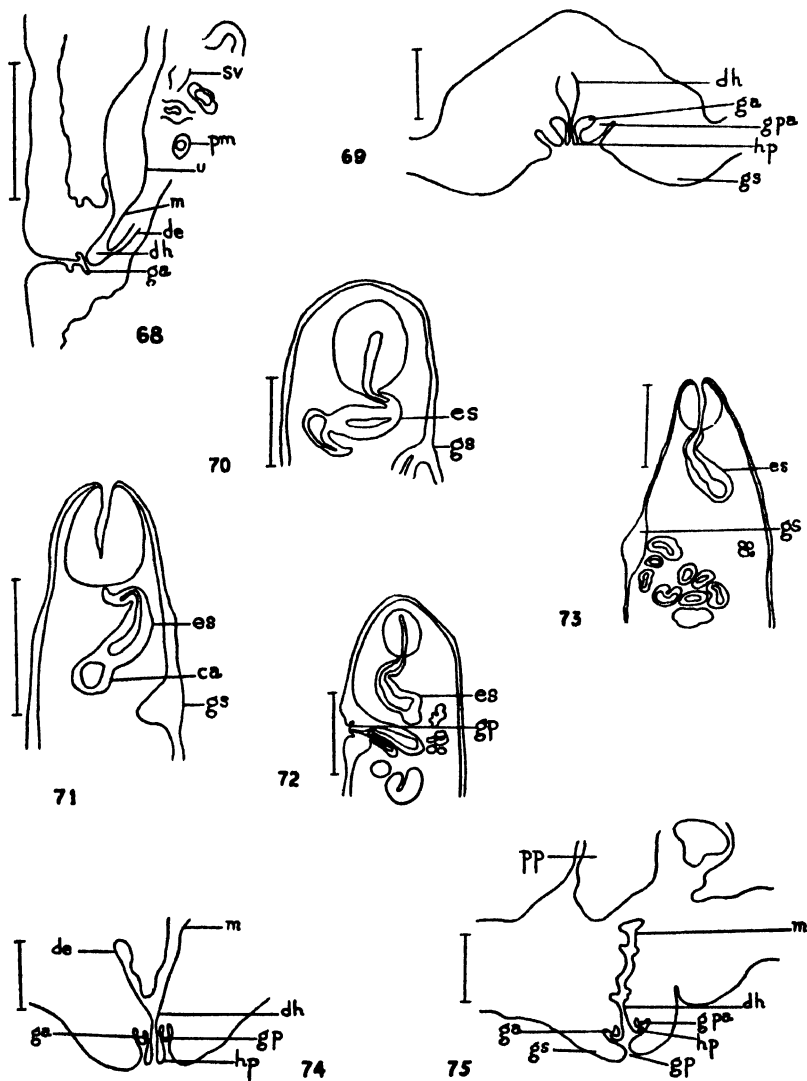


PLATE VIII

PLATE IX

FIG. 76.—Sagittal section of a specimen of migration size, 2.46×0.65 mm. Scale 1.0 mm.

FIG. 77.—Sagittal section of a very young specimen. Scale 1.0 mm.

FIG. 78.—Sagittal section of a mature specimen 2.8×1.26 mm. Scale 1.0 mm.

FIG. 79.—Frontal section of a mature specimen 2.99×1.61 mm. Scale 1.0 mm.

FIG. 80.—Graphic reconstruction of a mature specimen. Scale 1.0 mm.

FIG. 81.—Sagittal section of a mature specimen. Scale 1.0 mm.

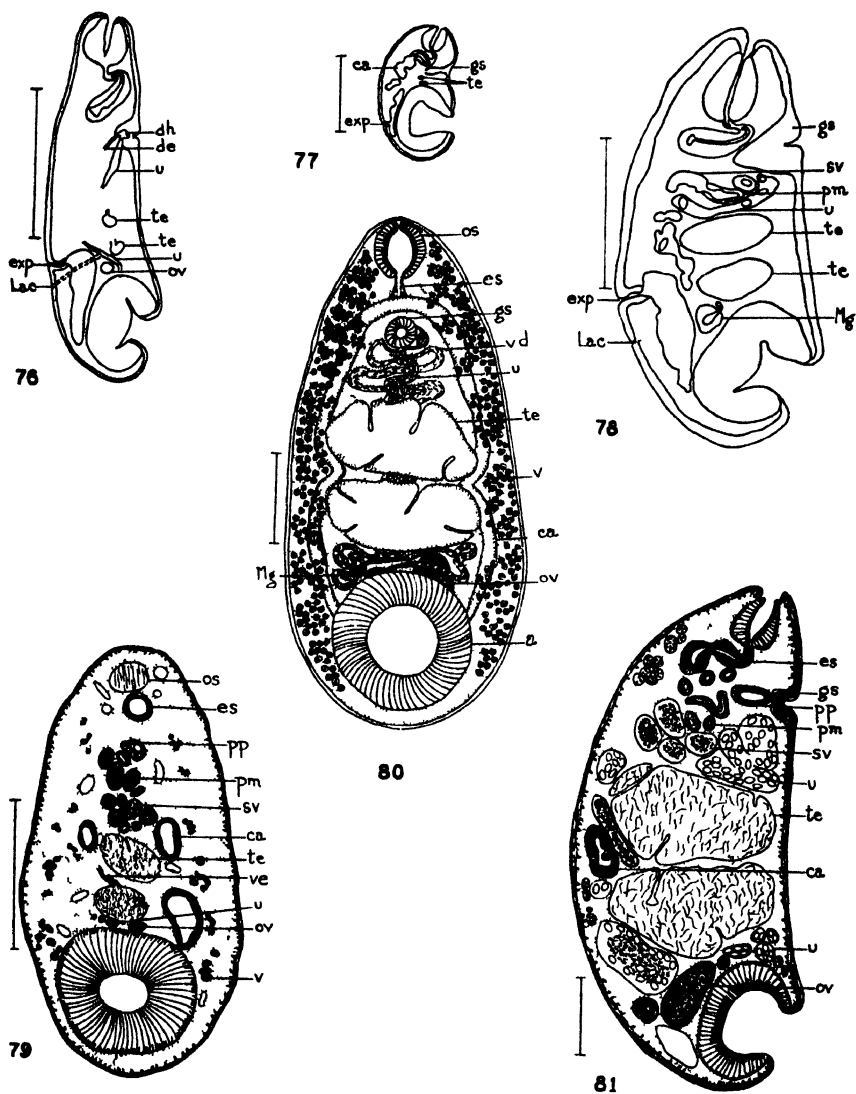


PLATE IX

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